

## 5. Anxiety and Stress

Preventative and curative options include:

Dietary changes, multivitamin and mineral formulas, extra calcium and magnesium, theanine, melatonin, DHEA, kava kava, green tea,

### **Piper methysticum (kava kava).**

Anon.

Altern Med Rev 1998 Dec;3(6):458-60

Piper methysticum (kava kava) is a plant native to the Pacific Island region, and has been used ceremonial for thousands of years. The active ingredients are a group of substances known as kava lactones (AKA kava pyrones). Four lactones in kava have been found to have significant analgesic and anesthetic effects via non-opiate pathways. Kava's most popular application is as a natural anxiolytic, comparing favorably in several studies to a number of prescription medications, including benzodiazepines. CNS effects seem to be mediated by several mechanisms. Studies have been conflicting regarding its GABA-receptor-binding capacity, although this has been found to occur in some studies. In vitro kava has been found to block norepinephrine uptake. It also has some anti-convulsant capabilities, which appear to be mediated by Na<sup>+</sup> channel receptor sites. The therapeutic dosage is in the range of 50-70 mg kava lactones three times daily. The most common side effect, usually seen only with long-term, heavy usage of the herb, is a scaly skin rash called "kava dermatopathy." It has also been known to potentiate other medications such as barbiturates and Xanax.

### **Effect of green tea rich in gamma-aminobutyric acid on blood pressure of Dahl salt-sensitive rats.**

Abe Y, Umemura S, Sugimoto K, Hirawa N, Kato Y, Yokoyama N, Yokoyama T, Iwai J, Ishii M. Second Department of Internal Medicine, Yokohama City University School of Medicine, Japan.

Am J Hypertens 1995 Jan;8(1):74-9

gamma-Aminobutyric acid (GABA) is known to be involved in the regulation of blood pressure by modulating the neurotransmitter release in the central and peripheral sympathetic nervous systems. This study investigated the antihypertensive effect of green tea rich in GABA (GABA-rich tea) in young and old Dahl salt-sensitive (S) rats. GABA-rich tea was made by fermenting fresh green tea leaves under nitrogen gas. In experiment 1, 21 11-month-old rats, fed a 4% NaCl diet for 3 weeks, were given water (group W), an ordinary tea solution (group T), or a GABA-rich tea solution (group G) for 4 weeks. The average GABA intake was 4.0 mg/rat per day. After 4 weeks of the treatment, blood

pressure was significantly decreased in group G (176 +/- 4; <.01) compared with group W (207 +/- 9) or group T (193 +/- 5 mm Hg). Plasma GABA levels were more elevated in group G (111 +/- 54) than in group W (not detectable) or group T (14 +/- 8 ng/mL; < .01 v G). In experiment 2, 21 5-week-old rats, fed a 4% NaCl diet, were divided into groups W, T, and G. The average GABA intake was 1.8 mg/rat per day. Body weight or chow and beverage consumption did not differ significantly among the three groups. After 4 weeks of the treatment, although blood pressure was comparable in groups W and T (165 +/- 3 v 164 +/- 5 mm Hg, mean +/- SE), it was significantly lower in group G (142 +/- 3 mm Hg) than in the other groups (< .01).(ABSTRACT TRUNCATED AT 250 WORDS)

### **Eleutherococcus senticosus (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look.**

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J Ethnopharmacol 2000 Oct;72(3):345-93

The adaptogen concept is examined from an historical, biological, chemical, pharmacological and medical perspective using a wide variety of primary and secondary literature. The definition of an adaptogen first proposed by Soviet scientists in the late 1950s, namely that an adaptogen is any substance that exerts effects on both sick and healthy individuals by 'correcting' any dysfunction(s) without producing unwanted side effects, was used as a point of departure. We attempted to identify critically what an adaptogen supposedly does and to determine whether the word embodies in and of itself any concept(s) acceptable to western conventional (allopathic) medicine. Special attention was paid to the reported pharmacological effects of the 'adaptogen-containing plant' *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae), referred to by some as 'Siberian ginseng', and to its secondary chemical composition. We conclude that so far as specific pharmacological activities are concerned there are a number of valid arguments for equating the action of so-called adaptogens with those of medicinal agents that have activities as anti-oxidants, and/or anti-cancerogenic, immunomodulatory and hypocholesteroletic as well as hypoglycemic and choleric action. However, 'adaptogens' and 'anti-oxidants' etc. also show significant dissimilarities and these are discussed. Significantly, the classical definition of an adaptogen has much in common with views currently being invoked to describe and explain the 'placebo effect'. Nevertheless, the chemistry of the secondary compounds of *Eleutherococcus* isolated thus far and their pharmacological effects support our hypothesis that the reported beneficial effects of adaptogens derive from their capacity to exert protective and/or inhibitory action against free radicals. An inventory of the secondary substances contained in *Eleutherococcus* discloses a potential for a wide range of activities reported from work on cultured cell lines, small laboratory animals and human subjects. Much of the cited work (although not all) has been published in peer-reviewed journals. Six compounds show various levels of activity as anti-oxidants, four show anti-cancer action, three show hypocholesterolemic activity,

two show immunostimulatory effects, one has choleric activity and one has the ability to decrease/moderate insulin levels, one has activity as a radioprotectant, one shows anti-inflammatory and anti-pyretic activities and yet another has shown activity as an antibacterial agent. Some of the compounds show more than one pharmacological effect and some show similar effects although they belong to different chemical classes. Clearly, Eleutherococcus contains pharmacologically active compounds but one wishes that the term adaptogen could be dropped from the literature because it is vague and conveys no insights into the mechanism(s) of action. If a precise action can be attributed to it, then the exact term for said action should obviously be used; if not, we strongly urge that generalities be avoided. Also, comparison of Eleutherococcus with the more familiar Panax ginseng C.A. Meyer (Araliaceae), 'true ginseng' has underscored that they differ considerably chemically and pharmacologically and cannot be justifiably considered as mutually interchangeable. Accordingly, we recommend that the designation 'Siberian ginseng' be dropped and be replaced with 'Eleutherococcus'. In the case of both Eleutherococcus and true ginseng, problems inherent in herbal preparation use include inconsistencies not only in terms of indications for use, but in the nomenclature of constituent chemical compounds, standardization, dosage and product labeling. (ABSTRACT TRUNCATED)

### **Inhibiting effects of theanine on caffeine stimulation evaluated by EEG in the rat.**

Kakuda T, Nozawa A, Unno T, Okamura N, Okai O. Central Research Institute, Itoen Ltd., Shizuoka, Japan. ITN00527@nifty.ne.jp

Biosci Biotechnol Biochem 2000 Feb;64(2):287-93

In this study, the inhibiting action of theanine on the excitation by caffeine at the concentration regularly associated with drinking tea was investigated using electroencephalography (EEG) in rats. First, the stimulatory action by caffeine i.v. administration at a level higher than 5 micromol/kg (0.970 mg/kg) b.w. was shown by means of brain wave analysis, and this level was suggested as the minimum dose of caffeine as a stimulant. Next, the stimulatory effects of caffeine were inhibited by an i.v. administration of theanine at a level higher than 5 micromol/kg (0.781 mg/kg) b.w., and the results suggested that theanine has an antagonistic effect on caffeine's stimulatory action at an almost equivalent molar concentration. On the other hand, the excitatory effects were shown in the rat i.v. administered 1 and 2 micromol/kg (0.174 and 0.348 mg/kg) b.w. of theanine alone. These results suggested two effects of theanine, depending on its concentration.

### **Exercise intensity and self-efficacy effects on anxiety reduction in healthy, older adults.**

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J Behav Med 1999 Jun;22(3):233-47

The purpose of the present study was to examine the effects of varying exercise intensities and changes in self-efficacy on anxiety reduction in a sample of healthy, older adults. Eighty older adults from a randomized controlled exercise trial participated in this study and completed measures of self-efficacy and the State Anxiety Inventory (SAI) prior to and following light-, moderate-, and high-intensity exercise. Latent growth curve modeling analyses revealed that although anxiety was reduced following the light-intensity condition, no significant changes in anxiety occurred following the moderate-intensity condition, and anxiety increased following the high-intensity condition. In addition, changes in self-efficacy were related to anxiety responses only in the moderate-intensity condition. An analysis of SAI items indicated that although the light-intensity condition resulted in decreased arousal and anxiousness, the high-intensity condition resulted in increased arousal and decreased anxiousness. These results are discussed in terms of social cognitive theory and the appropriateness of the SAI for use in exercise settings.

**The impact of a new emotional self-management program on stress, emotions, heart rate variability, DHEA and cortisol.**

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*Integr Physiol Behav Sci* 1998 Apr-Jun;33(2):151-70

This study examined the effects on healthy adults of a new emotional self-management program, consisting of two key techniques, "Cut-Thru" and the "Heart Lock-In." These techniques are designed to eliminate negative thought loops and promote sustained positive emotional states. The hypotheses were that training and practice in these techniques would yield lowered levels of stress and negative emotion and cortisol, while resulting in increased positive emotion and DHEA levels over a one-month period. In addition, we hypothesized that increased coherence in heart rate variability patterns would be observed during the practice of the techniques. Forty-five healthy adults participated in the study, fifteen of whom acted as a comparison group for the psychological measures. Salivary DHEA/DHEAS and cortisol levels were measured, autonomic nervous system function was assessed by heart rate variability analysis, and emotions were measured using a psychological questionnaire. Individuals in the experimental group were assessed before and four weeks after receiving training in the self-management techniques. The experimental group experienced significant increases in the positive affect scales of Caring and Vigor and significant decreases in the negative affect scales of Guilt, Hostility, Burnout, Anxiety and Stress Effects, while no significant changes were seen in the comparison group. There was a mean 23 percent reduction in cortisol and a 100 percent increase in DHEA/DHEAS in the experimental group. DHEA was significantly and positively related to the affective state Warmheartedness, whereas cortisol was significantly and positively related to Stress Effects. Increased coherence in heart rate variability patterns was measured in 80 percent of the experimental group during the use of the techniques. The results suggest that techniques designed to eliminate negative thought loops can have important positive effects on stress,

emotions and key physiological systems. The implications are that relatively inexpensive interventions may dramatically and positively impact individuals' health and well-being. Thus, individuals may have greater control over their minds, bodies and health than previously suspected.

**Scientific basis for the therapeutic use of *Withania somnifera* (ashwagandha): a review.**

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Altern Med Rev 2000 Aug;5(4):334-46

**OBJECTIVE:** The objective of this paper is to review the literature regarding *Withania somnifera* (ashwagandha, WS) a commonly used herb in Ayurvedic medicine. Specifically, the literature was reviewed for articles pertaining to chemical properties, therapeutic benefits, and toxicity.

**DESIGN:** This review is in a narrative format and consists of all publications relevant to ashwagandha that were identified by the authors through a systematic search of major computerized medical databases; no statistical pooling of results or evaluation of the quality of the studies was performed due to the widely different methods employed by each study.

**RESULTS:** Studies indicate ashwagandha possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoietic, and rejuvenating properties. It also appears to exert a positive influence on the endocrine, cardiopulmonary, and central nervous systems. The mechanisms of action for these properties are not fully understood. Toxicity studies reveal that ashwagandha appears to be a safe compound.

**CONCLUSION:** Preliminary studies have found various constituents of ashwagandha exhibit a variety of therapeutic effects with little or no associated toxicity. These results are very encouraging and indicate this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects. Clinical trials using ashwagandha for a variety of conditions should also be conducted.

**Efficacy of kava extract for treating anxiety: systematic review and meta-analysis.**

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J Clin Psychopharmacol 2000 Feb;20(1):84-9

Synthetic anxiolytic drugs are effective for treating anxiety, but they are burdened with adverse effects. Constraints on resources and time often render therapies

such as psychologic interventions impracticable. Thus, an effective oral medication with few adverse effects would be a welcome addition to the therapeutic repertoire. This systematic review and meta-analysis was aimed at assessing the evidence for or against the efficacy of kava extract as a symptomatic treatment for anxiety. Systematic literature searches were performed in the computerized databases MEDLINE, EMBASE, BIOSIS, AMED, CISCOM, and the Cochrane Library (all from their respective inception to June 1998). The search terms used were kava, kawa, kavain, Piper methysticum, and Rauschpfeffer (German term for Piper methysticum). Experts on the subject were contacted to provide further information. There were no restrictions regarding the language of publication. Double-blind, randomized, placebo-controlled trials of oral kava extract for the treatment of anxiety were included. All publications were blinded before assessment by a person not involved in the study. Data were extracted in a standardized, predefined fashion independently by the two reviewers. The methodologic quality of all trials was assessed. Superiority of kava extract over placebo was suggested by all seven reviewed trials. The meta-analysis of three trials suggests a significant difference in the reduction of the total score on the Hamilton Rating Scale for Anxiety in favor of kava extract (weighted mean difference, 9.69; 95% confidence interval, 3.54-15.83). These data imply that kava extract is superior to placebo as a symptomatic treatment for anxiety. Therefore, kava extract is an herbal treatment option for anxiety that is worthy of consideration.

#### **Coffee and tea intake and the risk of myocardial infarction.**

Sesso HD, Gaziano JM, Buring JE, Hennekens CH. Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02215, USA.

Am J Epidemiol 1999 Jan 15;149(2):162-7

The authors investigated the association of caffeinated coffee, decaffeinated coffee, and tea with myocardial infarction in a study of 340 cases and age-, sex-, and community-matched controls. The odds ratio for drinking  $\leq 4$  cups/day of caffeinated coffee versus drinking  $\leq 1$  cup/week was 0.84 (95% confidence interval (CI) 0.49-1.42) after adjustment for coronary risk factors (1 cup = 237 ml). The odds ratio for drinking  $\leq 1$  cup/day of decaffeinated coffee versus nondrinkers was 1.25 (95% CI 0.76-2.04). For tea, the odds ratio for drinking  $\leq 1$  cup/day versus nondrinkers was 0.56 (95% CI 0.35-0.90). In these data, only tea was associated with a lower risk of myocardial infarction.

#### **Effect of Korean red ginseng on psychological functions in patients with severe climacteric syndromes.**

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Int J Gynaecol Obstet 1999 Dec;67(3):169-74

**OBJECTIVE:** To evaluate the degree of psychological dysfunction and levels of stress hormones in postmenopausal women with climacteric syndromes and effect of Korean red ginseng (RG) on them.

**METHODS:** ACTH, cortisol and DHEA-S in peripheral blood from 12 postmenopausal women with climacteric syndromes or 8 postmenopausal women without any climacteric syndrome were measured before and 30 days after treatment with daily oral administration of 6 g RG. Blood samples were collected in the early morning on the bed-rest. In postmenopausal women with climacteric syndromes such as fatigue, insomnia and depression, psychological tests using the Cornell Medical Index (CMI) and the State-Trait Anxiety Inventory (STAI) were performed before and 30 days after treatment with RG.

**RESULTS:** CMI score as well as anxiety (A)-state in STAI score in postmenopausal women with climacteric syndromes was significantly higher than that without climacteric syndrome, while DHEA-S levels in postmenopausal women with climacteric syndromes were about a half of those without climacteric syndrome. Consequently, cortisol/DHEA-S (C/D) ratio was significantly higher in postmenopausal women with climacteric syndromes than in those without climacteric syndrome. When postmenopausal women with climacteric syndromes were treated with daily oral administration of 6 g RG for 30 days, CMI and STAI A-state scores decreased within normal range. Although the decreased DHEA-S levels were not restored to the levels in postmenopausal women without climacteric syndrome, the C/D ratio decreased significantly after treatment with RG.

**CONCLUSIONS:** Improvement of CMI and STAI scores in postmenopausal women suffering climacteric syndromes, particularly fatigue, insomnia and depression, by RG seemed to be brought about in part by effects of RG on stress-related hormones as shown by a decrease in C/D ratio.

**[Psychosomatic dysfunctions in the female climacteric. Clinical effectiveness and tolerance of Kava Extract WS 1490]** [Article in German]

Warnecke G. Gynakologe, Wuppertal.

Fortschr Med 1991 Feb 10;109(4):119-22

Within the framework of a randomized, placebo-controlled double-blind study, two groups each containing 20 patients with climacteric-related symptomatology were treated for a period of 8 weeks with kava WS 1490 extract 3 X 100 mg/day or a placebo preparation. The target variable - the HAMA overall score of anxiety symptomatology - revealed a significant difference in the drug-receiving group vis-a-vis the placebo group already after only 1 week of treatment. The course of such further parameters as depressive mood (DSI), subjective well-being (patient diary), severity of the disease (CGI), and the climacteric symptomatology (Kuppermann Index and Schneider scale) over the overall period of treatment demonstrate a high level of efficacy of kava extract WS 1490 in neurovegetative

and psychosomatic dysfunctions in the climacteric, associated with very good tolerance of the preparation.

**Treatment of anxiety patients. Double-blind study: Kava special extract WS 1490 versus benzodiazepine**

Woelk H.; Kapoula O.; Lehl S.; Schroter K.; Weinholz P. Psych./Akademisches Lehrkrankenhaus, Universitat Giessen, Licher Strasse 106,6300 Giessen Germany

Zeitschrift fur Allgemeinmedizin ( Z. ALLG.MED. ) (Germany) 1993, 69/10 (271-277)

No Abstract Available.

**Reduction effect of theanine on blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats.**

Yokogoshi H, Kato Y, Sagesaka YM, Takihara-Matsuura T, Kakuda T, Takeuchi N. School of Food and Nutritional Sciences, University of Shizuoka, Japan.

Biosci Biotechnol Biochem 1995 Apr;59(4):615-8

The effect of theanine, one of the components of green tea, on the blood pressure and brain 5-hydroxyindoles in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) was investigated by intraperitoneally administering theanine. The effect of glutamine, which is structurally similar to theanine, was also examined. When SHR were injected with various amounts of theanine (0, 500, 1000, 1500, and 2000 mg/kg), the change was dose-dependent, and a significant decrease in blood pressure was observed with the high doses (1500 and 2000 mg/kg). A dose of 2000 mg/kg of theanine did not alter the blood pressure of WKY, while the same dose to SHR decreased it significantly. On the other hand, glutamine administration to SHR did not change either the blood pressure or the heart rate. The brain 5-hydroxyindole level was significantly decreased by theanine administration to both WKY and SHR, the decrease being dose-dependent.

**Effect of theanine, r-glutamylethylamide, on brain monoamines and striatal dopamine release in conscious rats.**

Yokogoshi H, Kobayashi M, Mochizuki M, Terashima T. School of Food and Nutritional Sciences, The University of Shizuoka, Yada, Shizuoka, Japan.  
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Neurochem Res 1998 May;23(5):667-73

Theanine, r-glutamylethylamide, is one of the major components of amino acids in Japanese green tea. Effect of theanine on brain amino acids and monoamines, and the striatal release of dopamine (DA) was investigated. Determination of amino acids in the brain after the intragastric administration of theanine showed

that theanine was incorporated into brain through blood-brain barrier via leucine-preferring transport system. The concentrations of norepinephrine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindole acetic acid (5HIAA) in the brain regions were unaffected by the theanine administration except in striatum. Theanine administration caused significant increases in serotonin and/or DA concentrations in the brain, especially in striatum, hypothalamus and hippocampus. Direct administration of theanine into brain striatum by microinjection caused a significant increase of DA release in a dose-dependent manner. Microdialysis of brain with calcium-free Ringer buffer attenuated the theanine-induced DA release. Pretreatment with the Ringer buffer containing an antagonist of non-NMDA (N-methyl-D-aspartate) glutamate receptor, MK-801, for 1 hr did not change the significant increase of DA release induced by theanine. However, in the case of pretreatment with AP-5, (+/-)-2-amino-5-phosphonopentanoic acid; antagonist of NMDA glutamate receptor, the theanine-induced DA release from striatum was significantly inhibited. These results suggest that theanine might affect the metabolism and/or the release of some neurotransmitters in the brain, such as DA.

### **Cardiovascular risk factors among Japanese and American telephone executives.**

Anon.

Int J Epidemiol 1977 Mar;6(1):7-15

Cardiovascular risk factors were determined among two similar groups of telephone executives in Tokyo, Japan and New York City, USA. Both historical and electrocardiographic evidence pointed to a marked excess of coronary heart disease among American executives compared with their Japanese counterparts. In keeping with this finding, the Americans ate diets higher in animal fat, were fatter, and had higher serum cholesterol values but lower triglyceride levels. Mean blood pressures were slightly higher among the Japanese, and showed a greater increase with age. Urinary sodium/creatinine ratios were much higher among the Japanese, suggesting a higher salt intake. Cigarette smoking was more common among the Japanese. A review of other comparative studies between Japanese and Americans indicated that the only risk factors uniformly consistent with the frequency of coronary heart disease in the two countries were dietary fat, obesity, and serum cholesterol.

### **Cardiovascular risk factors in American and Japanese executives. Telecom Health Research Group.**

Comstock GW, Suzuki T, Stone RW, Crumrine JL, Johnson DH, Sakai Y, Matsuya T, Sasaki S.

J R Soc Med 1985 Jul;78(7):536-45

A standardized cardiovascular risk factor examination was given to executives in the headquarters of the American Telephone and Telegraph Company and the

Nippon Telegraph and Telephone Public Corporation. As expected from the national mortality data, evidence of ischaemic heart disease was more common among American than Japanese executives. The frequency of some but not all risk factors was consistent with the observed differences in ischaemic heart disease. Americans were fatter than their Japanese counterparts, obtained a higher proportion of their caloric intake from animal fats, had higher serum cholesterol levels, and more of them felt that their lives were highly stressful. On the other hand, Japanese executives were much more likely to be cigarette smokers and showed a greater increase in blood pressure with age. Serum high-density lipoprotein cholesterol levels and the ratio of saturated to unsaturated fatty acids in the serum were similar in the two groups.

**L theanine-a unique amino acid of green tea and its relaxation effect in humans.**

Juneja LR, et al. 1999.

Trends Food Sci Tech 10:199 204.

No abstract available.

**Protective effect of gamma-glutamylethylamide (theanine) on ischemic delayed neuronal death in gerbils.**

Kakuda T, Yanase H, Utsunomiya K, Nozawa A, Unno T, Kataoka K. Central Research Institute, Itoen, Ltd., Shizuoka, Japan. itn00527@nifty.ne.jp

Neurosci Lett 2000 Aug 11;289(3):189-92

We examined the protective effect of gamma-glutamylethylamide (theanine) on ischemic delayed neuronal death in field CA1 of the gerbil hippocampus. One microliter of theanine from each three concentrations (50, 125 and 500 microM) was administered through the lateral ventricle 30 min before ischemia. Transient forebrain ischemia was induced by bilateral occlusion of the common carotid arteries for 3 min under careful control of brain temperature at approximately 37 degrees C. Seven days after ischemia, the number of intact CA1 neurons in the hippocampus was assessed. Ischemia-induced neuronal death in hippocampal CA1 region was significantly prevented in a dose-dependent manner in the theanine-pretreated groups. These findings indicate that theanine might be useful clinically for preventing ischemic neuronal damage.

**Mortality among female practitioners of Chanoyu (Japanese "tea-ceremony").**

Sadakata S, Fukao A, Hisamichi S. Washiya Hospital, Utsunomiya.

Tohoku J Exp Med 1992 Apr;166(4):475-7

A cohort study aimed to evaluate the effect of drinking green tea on longevity was performed. Three thousand three hundred and eighty female practitioners of chanoyu (Japanese tea-ceremony), living in Tokyo, were followed from 1980 to 1988, and 280 were dead during this period. Standardized mortality ratios were estimated 0.55 when all Japanese women was used as standard population and 0.57 when women living in Tokyo was used, indicating the possibility that green tea is a protective factor for several fatal diseases.

**Health status and lifestyle in elderly Hawaii Japanese and Australian men. Exploring known differences in longevity.**

Simons LA, McCallum J, Simons J, Friedlander Y. University of NSW School of Medicine, Darlinghurst.

Med J Aust 1992 Aug 3;157(3):188-90

**OBJECTIVE:** To contrast health status and lifestyle in two elderly populations with differing longevity. **DESIGN:** Comparison of two cross-sectional data sets. **SETTING:** Non-institutionalised subjects. **SUBJECTS:** Men aged 60-81 years resident in Dubbo, New South Wales (n = 1183, 1988-1989) and Japanese men of the same ages resident in Hawaii (n = 1376, 1980-1982). **MAIN OUTCOME MEASURES:** Cardiovascular and non-cardiovascular disease prevalence, risk factors, social and health status.

**RESULTS:** A history of heart attack, angina and stroke was twice as prevalent in Dubbo men as in Hawaii Japanese. Other diseases were many times more prevalent in Dubbo--liver disease sixfold, prostate and renal disease twofold, and arthritis 1.5-fold. Hypercholesterolaemia and untreated hypertension were more prevalent in Dubbo (threefold and 1.5-fold respectively). Current smoking was similar in both groups, while diabetes was twice as prevalent in the Hawaii Japanese. More Dubbo men were widowed or lived alone, and fewer remained in paid employment. Dubbo men had more limited physical mobility.

**CONCLUSIONS:** Elderly Dubbo men have an excess of cardiovascular disease and associated risk factors, as well as an excess of non-cardiovascular disease, compared with Hawaii Japanese. This may account, in part, for a higher total mortality rate in elderly Australians compared with Japanese. Some of this disease burden may be amenable to risk factor intervention.

## 6. Arrhythmia (Cardiac)

Preventative and curative options include:

CoQ10, Perilla oil, flax oil, fish oil, Magnesium citrate, Potassium, Selenium, Acetyl-L-carnitine, Vitamin D3, Vitamin E, Calcium, Garlic, Ginkgo biloba, Olive leaf extract, Taurine, Thiamine, Tocotrienols, Vitamin E.

### **Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action**

Nair S.S.D.; Leitch J.W.; Falconer J.; Garg M.L.  
Australia  
Journal of Nutrition (USA), 1997, 127/3 (383-393)

The role of marine fish oil (n-3) polyunsaturated fatty acids in the prevention of fatal ventricular arrhythmia has been established in experimental animals. Prevention of arrhythmias arising at the onset of ischemia and reperfusion is important because if untreated, they result in sudden cardiac death. Animals supplemented with fish oils in their diet developed little or no ventricular fibrillation after ischemia was induced. Similar effects have also been observed in cultured neonatal cardiomyocytes. Several mechanisms have been proposed and studied to explain the antiarrhythmic effects of fish oil polyunsaturated fatty acids, but to date, no definite mechanism has been validated. The sequence of action of these mechanisms and whether more than one mechanism is involved is also not clear. Some of the mechanisms suggested to explain the antiarrhythmic action of fish oils include the incorporation and modification of cell membrane structure by (n-3) polyunsaturated fatty acids, their direct effect on calcium channels and cardiomyocytes and their role in eicosanoid metabolism. Other mechanisms that are currently being investigated include the role of (n-3) polyunsaturated fatty acids in cell signalling mediated through phosphoinositides and their effect on various enzymes and receptors. This article reviews these mechanisms and the antiarrhythmic studies using (n-3) polyunsaturated fatty acids.

### **Fatty acids suppress voltage-gated Na<sup>+</sup> currents in HEK293t cells transfected with the alpha-subunit of the human cardiac Na<sup>+</sup> channel**

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Proceedings of the National Academy of Sciences of the United States of America (United States), 1998, 95/5 (2680-2685)

Studies have shown that fish oils, containing n-3 fatty acids, have protective effects against ischemia-induced, fatal cardiac arrhythmias in animals and perhaps in humans. In this study we used the whole-cell voltage-clamp technique to assess the effects of dietary, free long-chain fatty acids on the Na<sup>+</sup> current (I(Na,α)) in human embryonic kidney (HEK293t) cells transfected with the α-subunit of the human cardiac Na<sup>+</sup> channel (hH1(α)). Extracellular application of 0.01 to 30 μM eicosapentaenoic acid (EPA, C20:5n-3) significantly reduced I(Na,α) with an IC<sub>50</sub> of 0.51 plus or minus 0.06 μM. The EPA-induced suppression of I(Na,α) was concentration- and voltage-dependent. EPA at 5 μM significantly shifted the steady-state inactivation relationship by -27.8 plus or minus 1.2 mV (n = 6, P < 0.0001) at the V(1/4) point. In addition, EPA blocked I(Na,α) with a higher 'binding affinity' to hH1(α) channels in the inactivated state than in the resting state. The transition from the resting state to the inactivated state was markedly accelerated in the presence of 5 μM EPA. The time for 50% recovery from the inactivation state was significantly slower in the presence of 5 μM EPA, from 2.1 plus or minus 0.8 ms for control to 34.8 plus or minus 2.1 ms (n = 5, P < 0.001). The effects of EPA on I(Na,α) were reversible. Furthermore, docosahexaenoic acid (C22:6n-3), α-linolenic acid (C18:3n-3), conjugated linoleic acid (C18:2n-7), and oleic acid (C18:1n-9) at 5 μM and all-trans-retinoic acid at 10 μM had similar effects on I(Na,α) as EPA. Even 5 μM of stearic acid (C18:0) or palmitic acid (C16:0) also significantly inhibited I(Na,α). In contrast, 5 μM EPA ethyl ester did not alter I(Na,α) (8 plus or minus 4%, n = 8, P > 0.05). The present data demonstrate that free fatty acids suppress I(Na,α) with high 'binding affinity' to hH1(α) channels in the inactivated state and prolong the duration of recovery from inactivation.

### **n-3 Polyunsaturated fatty acids, heart rate variability and ventricular arrhythmias in patients with previous myocardial infarcts**

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Ugeskrift for Laeger (Denmark), 1997, 159/37 (5525-5529)

There is evidence for an antiarrhythmic effect of n-3 polyunsaturated fatty acids (n-3 PUFA) in animals. The aim of the present study was to investigate the effect of dietary n-3 PUFA on ventricular arrhythmias and heart rate variability (HRV) in patients with a previous myocardial infarction. Fifty-five patients were randomized to receive either 5.2 g of n-3 PUFA daily for 12 weeks or placebo in a double blind, placebo-controlled study. Prior to randomization a 24-hour Holter recording was obtained, and this was repeated at the end of the study. The major end-points were the number of ventricular extrasystoles (VE)/24 hours and the 24-hour HRV. A non-significant decrease in VE/24 hours was found in both the n-3 PUFA group and among controls after dietary supplementation, whereas HRV significantly increased after n-3 PUFA compared to both baseline values (p =

0,04) and to controls ( $p = 0,01$ ). The present study therefore supports the hypothesis that n-3 PUFA may have an antiarrhythmic effect in humans.

#### **Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: The Indian experiment of infarct survival - 4**

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Cardiovascular Drugs and Therapy (USA), 1997, 11/3 (485-491)

In a randomized, placebo-controlled trial, the effects of treatment with fish oil (eicosapentaenoic acid, 1.08 g/day) and mustard oil (alpha-linolenic acid, 2.9 g/day) were compared for 1 year in the management of 122 patients (fish oil, group A), 120 patients (mustard oil, group B), and 118 patients (placebo, group C) with suspected acute myocardial infarction (AMI). Treatments were administered about (mean) 18 hours after the symptoms of AMI in all three groups. The extent of cardiac disease, rise in cardiac enzymes, and lipid peroxides were comparable among the groups at entry into the study. After 1 year total cardiac events were significantly less in the fish oil and mustard oil groups compared with the placebo group (24.5% and 28% vs. 34.7%,  $p < 0.01$ ). Nonfatal infarctions were also significantly less in the fish oil and mustard oil groups compared with the placebo group (13.0% and 15.0% vs. 25.4%,  $p < 0.05$ ). Total cardiac deaths showed no significant reduction in the mustard oil group; however, the fish oil group had significantly less cardiac deaths compared with the placebo group (11.4% vs. 22.0%,  $p < 0.05$ ). Apart from the decrease in the cardiac event rate, the fish oil and mustard oil groups also showed a significant reduction in total cardiac arrhythmias, left ventricular enlargement, and angina pectoris compared with the placebo group. Reductions in blood lipoproteins in the two intervention groups were modest and do not appear to be the cause of the benefit in the two groups. Diene conjugates showed a significant reduction in the fish oil and mustard oil groups, indicating that a part of the benefit may be caused by the reduction in oxidative stress. The findings of this study suggest that fish oil and mustard oil, possibly due to the presence of n-3 fatty acids, may provide rapid protective effects in patients with AMI. However, a large study is necessary to confirm this suggestion.

#### **omega3 fatty acids in the prevention-management of cardiovascular disease**

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Canadian Journal of Physiology and Pharmacology (Canada), 1997, 75/3 (234-239)

Epidemiologic studies show that populations who eat fish versus those who do not have a reduced death rate from cardiovascular disease. Experimental studies have shown that omega-3 fatty acids affect the function of cells involved in atherothrombosis in numerous ways, including the modification of eicosanoid products in the cyclooxygenase and lipoxygenase pathways, the reduced synthesis of cytokines and platelet-derived growth factor, and alterations of leukocyte and endothelial cell properties. Intervention studies in patients with restenosis, myocardial infarction, and cardiac arrhythmias with omega-3 fatty acid supplementation have been addressed in several clinical studies. The ingestion of omega-3 fatty acids following one episode of myocardial infarction appears to decrease the rate of cardiac death. These effects of omega-3 fatty acids appear to be due to their antiarrhythmic properties. In fact, fish oil has been shown to reduce ventricular arrhythmias and to be more beneficial than currently used pharmacologic agents. The dose, duration, and mechanisms involved in the prevention and management of cardiovascular disease following omega-3 fatty acid ingestion or supplementation need to be investigated by double blind controlled clinical trials.

### **Omega-3 fatty acids and prevention of cardiovascular disease**

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Cahiers de Nutrition et de Dietetique (France), 1997, 32/2 (107-114)

Most of the cardio-vascular disease (CVD) risk factors may be controlled by nutrition. Polyunsaturated fatty acids (PUFA) of the omega3 series are known for their beneficial effect on risk, but could also influence the CVD severity through their action on the heart, very sensitive to diet-induced alterations of membrane composition. Introducing omega3 PUFA in the diet results in an inversion of the AA/DHA ratio, mainly due to an increase in DHA content. In several experimental models, such structural changes were reported to affect cardiac functions. Arrhythmia which occurs during ischemia and reperfusion, is largely reduced when the membrane contains 20% DHA. Moreover, the membrane omega3 PUFA appear to increase energy utilization efficiency. This may be related to the positive effect of fish oil on the decrease of heart rate in rat in vivo, and on the recovery of mitochondrial function in the post-ischemic heart. At a more cellular level, the omega3 PUFAs (particularly DHA) can influence the activity of phospholipase A2, which contributes to membrane homeostasis, the prostaglandin production or the function of adrenergic receptors, a key system in the regulation of cardiac activity. Quite similar effects were reported in pathological conditions since the presence of omega3 PUFAs in the membranes enhances the cellular recovery after hypoxia and blocks the stimulation of prostacycline synthesis induced by post-hypoxic reoxygenation. However, much research remains to be done, in order to understand the interactions between diet-induced membrane alterations and cardiac physiology, pathology, and pharmacology.

### **Vitamin E analogues reduce the incidence of ventricular fibrillations and scavenge free radicals**

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Fundamental and Clinical Pharmacology (France), 1998, 12/2 (164-172)

The aim of our study was to analyse the protective effects of different alpha-tocopherol analogues 1) against fibrillations induced by an ischemia-reperfusion sequence, and 2) to further investigate in vitro the radical scavenging properties of these analogues by two sensitive methods. Concerning 1: isolated rat hearts underwent 10 min of coronary ligation followed by reperfusion and the alpha-tocopherol analogues were infused 15 min before occlusion. Functional parameters including heart rate and fibrillations were recorded. Concerning 2: the beta-phycoerythrin assay was utilised to determine the oxygen radical absorbing capacity: (ORAC) of these vitamin E analogues against peroxy radicals. Electron paramagnetic resonance (EPR) was used to measure their scavenger abilities on hydroxyl radical and superoxide anion production. Concerning 1: ventricular fibrillation times were reduced for all analogues treated hearts at concentrations of 1 microM and 5 microM, with Trolox being the most efficacious. Concerning 2: in our experimental conditions of intense production of free radicals, scavenging IC50 values for hydroxyl radical were 1.15, 2.17 and 4.04 mM for Trolox, MDL 74270 and MDL 74366 respectively. Superoxide anion IC50 values were 1.0 and 6.75 mM for Trolox and MDL 74270. Our results show that water-soluble analogues of vitamin E are effective in the prevention of coronary ligation induced reperfusion arrhythmia under our experimental conditions. Moreover, our data demonstrate that these vitamin E analogues are effective scavengers for a variety of radicals. Our studies support the view that compounds that can either inhibit the formation or scavenge free radicals can protect the heart against arrhythmia associated with ischemia-reperfusion.

### **Antioxidant activity of U-83836E, a second generation lazaroid, during myocardial Ischemia/Reperfusion injury**

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Free Radical Research (United Kingdom), 1997, 27/6 (577-590)

The 21-aminosteroid compounds are potent lipid per oxidation inhibitors belonging to a new class of antioxidants given the collective name of 'lazaroids'. They protect cells from oxidative damage induced by oxygen-based free radicals in a variety of in vitro and in vivo test systems. U-83836E is one of the second-

generation lazaroids that are based on a non steroidal structure characterized by a ring portion of alpha-tocopherol bonded with various amine groups. We investigated the ability of U-83836E to reduce myocardial damage in rats undergoing left coronary artery occlusion for 60 min followed by 6 hours of reperfusion. This ischemia/reperfusion model produced wide heart necrosis, membrane lipid peroxidation, ventricular arrhythmias, tissue neutrophil infiltration and a marked decrease in endogenous antioxidants. Intravenous administration of U-83836E, (7.5, 15 and 30 mg/kg) at onset of reperfusion, reduced myocardial necrosis, expressed as a percentage of either the area at risk or the total left ventricle ( $p < 0.001$ ), improved haemodynamic conditions by decreasing ventricular arrhythmias ( $p < 0.005$ ), limited membrane lipid peroxidation (evaluated by assessing conjugated dienes,  $p < 0.001$ ; and 4-hydroxy-nonenal,  $p < 0.001$ ) restored the endogenous antioxidants vitamin E ( $p < 0.001$ ), and superoxide dismutase ( $p < 0.001$ ). Furthermore, the lazaroid inhibited the derimental hydroxyl radical formation ( $p < 0.001$ ), evaluated indirectly by a trapping agent and reduced heart neutrophil infiltration, measured by testing cardiac tissue elastase ( $p < 0.001$ ) that is released from the stimulated granulocytes at the site of injury. These data suggest that this compound could be a new useful tool to study the mechanisms of oxidative damage during myocardial infarction.

### **Trace elements and cardioprotection: Increasing endogenous glutathione peroxidase activity by oral selenium supplementation in rats limits reperfusion-induced arrhythmias**

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Journal of Trace Elements in Medicine and Biology (Germany), 1998, 12/1 (28-38)

Oxyradicals have been implicated as a possible cause of reperfusion- arrhythmias (RA). However, the use of diverse exogenous oxyradical scavengers designed to reduce RA has given contradictory results. The aim of the present study was to determine whether enhancing the activity of the main endogenous enzyme involved in peroxide elimination in cardiac cells, namely glutathione peroxidase, may limit RA in isolated heart preparations by increasing their antioxidant status. For this purpose, a group of 15 male Wistar rats received a selenium enriched diet for ten weeks (1.5 mg Se/kg diet). Control animals (n=15) received a standard diet containing 0.05 mg Se/kg diet. The incidence of early ventricular arrhythmias was investigated during the reperfusion period following 10 min regional ischemia induced ex-vivo by left coronary artery ligation. Our results show that selenium-supplementation significantly increased the global selenium status of the animals. In the isolated heart preparations, the selenium supplementation induced a significant reduction of the severity of RA as assessed by the arrhythmia score and the limitation of the incidence of both ventricular tachycardia (control: 91% vs, selenium: 36%,  $p < 0.05$ ) and irreversible ventricular fibrillation (control: 45%

vs selenium: 0%,  $p < 0.05$ ). These effects were associated with a significant increase in cardiac mitochondrial and cytosolic glutathione peroxidase activities in both the left and the right ventricles. These results illustrate the potential protective effect of selenium against ischemia- reperfusion injury and suggest that peroxides might play a key role in the genesis of some aspects of the reperfusion syndrome.

### **Randomized, double-blind placebo-controlled trial of coenzyme Q10 in patients with acute myocardial infarction**

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Cardiovascular Drugs and Therapy (United States), 1998, 12/4 (347-353)

The effects of oral treatment with coenzyme Q10 (120 mg/d) were compared for 28 days in 73 (intervention group A) and 71 (placebo group B) patients with acute myocardial infarction (AMI). After treatment, angina pectoris (9.5 vs. 28.1), total arrhythmias (9.5% vs. 25.3%), and poor left ventricular function (8.2% vs. 22.5%) were significantly ( $P < 0.05$ ) reduced in the coenzyme and group than placebo group. Total cardiac events, including cardiac deaths and nonfatal infarction, were also significantly reduced in the coenzyme Q10 group compared with the placebo group (15.0% vs. 30.9%,  $P < 0.02$ ). The extent of cardiac disease, elevation in cardiac enzymes, and oxidative stress at entry to the study were comparable between the two groups. Lipid peroxides, diene conjugates, and malondialdehyde, which are indicators of oxidative stress, showed a greater reduction in the treatment group than in the placebo group. The antioxidants vitamin A, E, and C and beta-carotene, which were lower initially after AMI, increased more in the coenzyme Q10 group than in the placebo group. These findings suggest that coenzyme Q10 can provide rapid protective effects in patients with AMI if administered within 3 days of the onset of symptoms. More studies in a larger number of patients and long-term follow-up are needed to confirm our results.

### **Effect of coenzyme Q10 therapy in patients with congestive heart failure: A long-term multicenter randomized study**

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Clin. Invest. Suppl. (Germany), 1993, 71/8 (S 134-S 136)

The improved cardiac function in patients with congestive heart failure treated with coenzyme Q10 supports the hypothesis that this condition is characterized by mitochondrial dysfunction and energy starvation, so that it may be ameliorated by coenzyme Q10 supplementation. However, the main clinical problems in patients with congestive heart failure are the frequent need of hospitalization and the high

incidence of life-threatening arrhythmias, pulmonary edema, and other serious complications. Thus, we studied the influence of coenzyme Q10 long-term treatment on these events in patients with chronic congestive heart failure (New York Heart Association functional class III and IV) receiving conventional treatment for heart failure. They were randomly assigned to receive either placebo (n = 322, mean age 67 years, range 30-88 years) or coenzyme Q10 (n = 319, mean age 67 years, range 26-89 years) at the dosage of 2 mg/kg per day in a 1-year double-blind trial. The number of patients who required hospitalization for worsening heart failure was smaller in the coenzyme Q10 treated group (n = 73) than in the control group (n = 118, P < 0.001). Similarly, the episodes of pulmonary edema or cardiac asthma were reduced in the control group (20 versus 51 and 97 versus 198, respectively; both P < 0.001) as compared to the placebo group. Our results demonstrate that the addition of coenzyme Q10 to conventional therapy significantly reduces hospitalization for worsening of heart failure and the incidence of serious complications in patients with chronic congestive heart failure.

**Serum concentration of lipoprotein(a) decreases on treatment with hydrosoluble coenzyme Q10 in patients with coronary artery disease: discovery of a new role.**

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Int J Cardiol 1999 Jan;68(1):23-9

**OBJECTIVE:** To examine the effect of coenzyme Q10 supplementation on serum lipoprotein(a) in patients with acute coronary disease.

**STUDY DESIGN:** Randomized double blind placebo controlled trial.

**SUBJECTS AND METHODS:** Subjects with clinical diagnosis of acute myocardial infarction, unstable angina, angina pectoris (based on WHO criteria) with moderately raised lipoprotein(a) were randomized to either coenzyme Q10 as Q-Gel (60 mg twice daily) (coenzyme Q10 group, n=25) or placebo (placebo group, n=22) for a period of 28 days.

**RESULTS:** Serum lipoprotein(a) showed significant reduction in the coenzyme Q10 group compared with the placebo group (31.0% vs 8.2% P<0.001) with a net reduction of 22.6% attributed to coenzyme Q10. HDL cholesterol showed a significant increase in the intervention group without affecting total cholesterol, LDL cholesterol, and blood glucose showed a significant reduction in the coenzyme Q10 group. Coenzyme Q10 supplementation was also associated with significant reductions in thiobarbituric acid reactive substances, malon/dialdehyde and diene conjugates, indicating an overall decrease in oxidative stress.

**CONCLUSION:** Supplementation with hydrosoluble coenzyme Q10 (Q-Gel) decreases lipoprotein(a) concentration in patients with acute coronary disease.

### **Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects.**

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Proc Natl Acad Sci U S A 1998 Jul 21;95(15):8892-7

Coenzyme Q10 is an essential cofactor of the electron transport chain as well as a potent free radical scavenger in lipid and mitochondrial membranes. Feeding with coenzyme Q10 increased cerebral cortex concentrations in 12- and 24-month-old rats. In 12-month-old rats administration of coenzyme Q10 resulted in significant increases in cerebral cortex mitochondrial concentrations of coenzyme Q10. Oral administration of coenzyme Q10 markedly attenuated striatal lesions produced by systemic administration of 3-nitropropionic acid and significantly increased life span in a transgenic mouse model of familial amyotrophic lateral sclerosis. These results show that oral administration of coenzyme Q10 increases both brain and brain mitochondrial concentrations. They provide further evidence that coenzyme Q10 can exert neuroprotective effects that might be useful in the treatment of neurodegenerative diseases.

### **Magnesium in supraventricular and ventricular arrhythmias**

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Zeitschrift fur Kardiologie (Germany), 1996, 85/Suppl. 6 (135-145)

The use of magnesium as an antiarrhythmic agent in ventricular and supraventricular arrhythmias is a matter of an increasing but still controversial discussion during recent years. With regard to the well established importance of magnesium in experimental studies for preserving electrical stability and function of myocardial cells and tissue, the use of magnesium for treating one or the other arrhythmia seems to be a valid concept. In addition, magnesium application represents a physiologic approach, and by this, is simple, cost-effective and safe for the patient. However, when one reviews the available data from controlled studies on the antiarrhythmic effects of magnesium, there are only a few types of diac arrhythmias, such as torsade de pointes, digitalis-induced ventricular arrhythmias and ventricular arrhythmias occurring in the presence of heart failure or during the perioperative state, in which the antiarrhythmic benefit of magnesium has been shown and/or established. Particularly in patients with one of these types of cardiac arrhythmias, however, it should be realized that preventing the patient from a magnesium deficit is the first, and the application of magnesium the second best strategy to keep the patient free from cardiac arrhythmias.

### **Effect of intravenous magnesium sulfate on cardiac arrhythmias in critically ill patients with low serum ionized magnesium**

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Japanese Circulation Journal (Japan), 1996, 60/11 (871-875)

Magnesium affects cardiac function, although until the recent development of a new ion selective electrode no method existed for measuring the physiologically active form of magnesium, free ions ( $iMg^{2+}$ ), in the blood. We investigated the antiarrhythmic effect of magnesium sulfate administered to critically ill patients with cardiac arrhythmias and reduced  $iMg^{2+}$  as determined using the ion-selective electrode. Eight patients with a low  $iMg^{2+}$  level (less than 0.40 mmol/L) were given intravenous magnesium sulfate (group L). Magnesium sulfate was also administered to patients with a normal  $iMg^{2+}$  level (more than 0.40 mmol/L) but who did not respond to conventional antiarrhythmic drugs (group N). Intravenous magnesium sulfate significantly increased the  $iMg^{2+}$  level in patients in group L from 0.35 plus or minus 0.06 mmol/L (mean plus or minus SD) to 0.54 plus or minus 0.09 mmol/L ( $p < 0.01$ ), and had an antiarrhythmic effect in 7 of the 8 patients (88%). However, in group N patients, intravenous magnesium sulfate had an antiarrhythmic effect in only 1 of the 6 patients (17%) ( $p < 0.05$  vs group L). These results suggest that intravenous magnesium sulfate may be effective in the acute management of cardiac arrhythmias in patients with a low serum  $iMg^{2+}$  level.

### **Prophylactic effects of taurine and diltiazem, alone or combined, on reperfusion arrhythmias in rats**

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*Acta Pharmacologica Sinica* (China), 1996, 17/2 (122-124)

**Aim:** To study the effects of taurine (Tau) and diltiazem (Dil), alone or in combination, on reperfusion arrhythmias in anesthetized rats.

**Methods:** The arrhythmias were produced by coronary artery ligation for 15 min followed by reperfusion. Malondialdehyde (MDA) content and superoxide dismutase (SOD) activity were measured by thiobarbituric acid fluorescence assay and colorimetric determination.

**Results:** Taurine 70 mg . kg<sup>-1</sup> in combination with Dil 1 mg . kg<sup>-1</sup> were more effective on prevention of the reperfusion arrhythmias than each drug alone. The combination of both drugs not only decreased the content of MDA, but also increased the activity of SOD in reperfusion myocardium.

Conclusion: The inhibition of lipoperoxides formation as well as the inhibition of the calcium influx was involved in the anti-arrhythmic effect of both taurine and diltiazem.

### **The cardiovascular protective role of docosahexaenoic acid**

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European Journal of Pharmacology (Netherlands), 1996, 300/1-2 (83-89)

Dietary fish oils rich in n-3 polyunsaturated fatty acids can modulate a diverse range of factors contributing to cardiovascular disease. This study examined the relative roles of eicosapentaenoic acid (20:5 n-3; EPA) and docosahexaenoic acid (22:6 n-3; DHA) which are the principal n-3 polyunsaturated fatty acids regarded as candidates for cardioprotective actions. At low dietary intakes (0.4-1.1% of energy (%en)), docosahexaenoic acid but not eicosapentaenoic acid inhibited ischaemia-induced cardiac arrhythmias. At intakes of 3.9-10.0%en, docosahexaenoic acid was more effective than eicosapentaenoic acid at retarding hypertension development in spontaneously hypertensive rats (SHR) and inhibiting thromboxane-like vasoconstrictor responses in aortas from SHR. In stroke-prone SHR with established hypertension, docosahexaenoic acid (3.9-10.0%en) retarded the development of salt-loading induced proteinuria but eicosapentaenoic acid alone was ineffective. The results demonstrate that purified n-3 polyunsaturated fatty acids mimic the cardiovascular actions of fish oils and imply that docosahexaenoic acid may be the principal active component conferring cardiovascular protection.

### **Trace elements in prognosis of myocardial infarction and sudden coronary death**

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Journal of Trace Elements in Experimental Medicine (USA), 1996, 9/2 (57-62)

Ca, Cu, Mg, Mn, and Zn concentrates were measured in plasma, RBC, and hair of 350 men aged 40-59 years with myocardial infarction (MI) and/or who died from sudden cardiac death (SCD), as compared with normal controls. Analyses were done by flame atomic absorption spectrophotometry. Cu in plasma of MI patients was significantly higher than the controls'. Plasma Mn was significantly lower in SCD than in MI subjects. No other consistent and significant changes were observed. Past and present evidence indicates that high plasma Cu levels may be associated with heart failure and rhythm disorders. The low plasma Mn levels may be an indicator of decreased parasympathetic tonus thus favouring

myocardial desynchronization and A-V block. Cu inhibits phosphodiesterase activity and Mn inhibits adenylate cyclase activity thus exerting an influence on the contractility of cardiomyocytes and of smooth muscle cells in coronary arteries. Cu and Mn analyses may thus have a prognostic significance for MI and SCD.

### **Prevention of cardiac arrhythmia by dietary (n-3) polyunsaturated fatty acids and their mechanism of action**

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Journal of Nutrition (USA), 1997, 127/3 (383-393)

The role of marine fish oil (n-3) polyunsaturated fatty acids in the prevention of fatal ventricular arrhythmia has been established in experimental animals. Prevention of arrhythmias arising at the onset of ischemia and reperfusion is important because if untreated, they result in sudden cardiac death. Animals supplemented with fish oils in their diet developed little or no ventricular fibrillation after ischemia was induced. Similar effects have also been observed in cultured neonatal cardiomyocytes. Several mechanisms have been proposed and studied to explain the antiarrhythmic effects of fish oil polyunsaturated fatty acids, but to date, no definite mechanism has been validated. The sequence of action of these mechanisms and whether more than one mechanism is involved is also not clear. Some of the mechanisms suggested to explain the antiarrhythmic action of fish oils include the incorporation and modification of cell membrane structure by (n-3) polyunsaturated fatty acids, their direct effect on calcium channels and cardiomyocytes and their role in eicosanoid metabolism. Other mechanisms that are currently being investigated include the role of (n-3) polyunsaturated fatty acids in cell signalling mediated through phosphoinositides and their effect on various enzymes and receptors. This article reviews these mechanisms and the antiarrhythmic studies using (n-3) polyunsaturated fatty acids.

### **Exposure to the n-3 polyunsaturated fatty acid docosahexaenoic acid impairs alpha1-adrenoceptor-mediated contractile responses and inositol phosphate formation in rat cardiomyocytes**

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Naunyn-Schmiedeberg's Archives of Pharmacology (Germany), 1996, 354/2 (109-119)

The beneficial effects of n-3 polyunsaturated fatty acids of fish oil in the prevention of fatal arrhythmias in myocardial ischemia were suggested to be at

least in part mediated by a modulation of dihydropyridine-sensitive L-type calcium channels. As cardiac alpha1-adrenoceptor stimulation has been suggested to have no significant effect on L-type calcium channels, the aim of this study using cultured neonatal rat cardiomyocytes was to investigate whether chronic n-3 polyunsaturated fatty acid exposure may have an influence on alpha1-adrenoceptor-induced positive inotropic effects and induction of arrhythmias. Pretreatment of the rat cardiomyocytes for 3 days in the presence of the n-3 polyunsaturated fish oil-derived fatty acid docosahexaenoic acid (60 micromol/l) markedly decreased alpha1-adrenoceptor-stimulated increase in contraction velocity and induction of arrhythmias. The increase in contraction velocity of the cardiomyocytes induced by the beta-adrenoceptor agonist isoprenaline was also markedly reduced by the n-3 fatty acid pretreatment. Basal contractile amplitude and spontaneous beating frequency of the cardiomyocytes were not significantly altered by the docosahexaenoic acid exposure. The pretreatment of the rat cardiomyocytes for 3 days in the presence of docosahexaenoic acid (60 micromol/l) decreased alpha1-adrenoceptor-stimulated formation of the calcium-mobilizing second messenger IP3 and its metabolites IP2 and IP1 by 55%. The depression of IP3 formation by docosahexaenoic acid treatment was not mediated by a decreased uptake of myo-inositol into the cardiomyocytes nor by a decreased synthesis of phosphatidylinositol bisphosphate (PIP2), the substrate of phospholipase C. The level of glycerol-3-phosphate, an important substrate of the phosphoinositide cycle, was unaltered by the docosahexaenoic acid pretreatment. Receptor binding studies revealed that the dissociation constant and maximal binding capacity of the alpha1-adrenoceptor antagonist (3H)prazosin was unchanged by the n-3 polyunsaturated fatty acid exposure. beta-Adrenoceptor- and forskolin-stimulated adenylyl cyclase activities were not diminished by the docosahexaenoic acid pretreatment. Chronic exposure of the cardiomyocytes to the n-6 polyunsaturated fatty acid arachidonic acid (60 micromol/l) did neither significantly alter alpha1-adrenoceptor-induced inositol phosphate formation nor alpha1-adrenoceptor-stimulated increase in contraction velocity. The results presented show that chronic n-3 polyunsaturated fatty acid pretreatment of rat cardiomyocytes leads to a marked impairment of alpha1-adrenoceptor-induced positive inotropic effects and induction of arrhythmias concomitant with a n-3 fatty acid-induced decrease in IP3 formation. This derangement of the phosphoinositide pathway by chronic n-3 fatty acid exposure may, thus, contribute to the beneficial effects of fish oil-derived fatty acids in the prevention of fatal arrhythmias in myocardial ischemia.

### **Selenium deficiency associated with cardiac dysfunction in three patients with chronic respiratory failure**

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Japanese Journal of Thoracic Diseases (Japan), 1996, 34/12 (1406-1410)

We encountered three patients with chronic respiratory failure who had heart failure of cardiac arrhythmias and low levels of serum selenium. All three had

tracheostomies and had received long-term parenteral nutrition that had not included selenium. All three also had refractory cardiac dysfunction, which was manifested in edema, heart failure, and various tachycardias. We suspected that selenium deficiency had caused their cardiac dysfunction. Serum selenium concentrations were found to be much lower than normal in all three, so 100 microg/day of selenium was administered in addition to their tube feedings. Cardiac function improved after replacement of selenium. These cases show the need for preventing selenium deficiency in patients with chronic respiratory failure during long-term administration of parenteral nutrition.

### **Fish oil and other nutritional adjuvants for treatment of congestive heart failure**

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Medical Hypotheses (United Kingdom), 1996, 46/4 (400-406)

Published clinical research, as well as various theoretical considerations, suggest that supplemental intakes of the 'metavitamins' taurine, coenzyme Q10, and L-carnitine, as well as of the minerals magnesium, potassium, and chromium, may be of therapeutic benefit in congestive heart failure. High intakes of fish oil may likewise be beneficial in this syndrome. Fish oil may decrease cardiac afterload by an antivasopressor action and by reducing blood viscosity, may reduce arrhythmic risk despite supporting the heart's beta-adrenergic responsiveness, may decrease fibrotic cardiac remodeling by impeding the action of angiotensin II and, in patients with coronary disease, may reduce the risk of atherothrombotic ischemic complications. Since the measures recommended here are nutritional and carry little if any toxic risk, there is no reason why their joint application should not be studied as a comprehensive nutritional therapy for congestive heart failure.

### **Evidence on the participation of the 3',5'-cyclic AMP pathway in the non-genomic action of 1,25-dihydroxy-vitamin D3 in cardiac muscle.**

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Mol Cell Endocrinol (Netherlands) Dec 1991, 82 (2-3) p229-35

Several studies have suggested that vitamin D plays a role in cardiovascular function. It has been recently shown that in vitro treatment of vitamin D-deficient chick cardiac muscle with physiological concentrations of 1,25-dihydroxy-vitamin D3 (1,25(OH)2D3) induces a rapid (1-10 min) increase of tissue <sup>45</sup>Ca uptake which can be suppressed by Ca channel blockers. The hormone simultaneously stimulated heart microsomal membrane protein phosphorylation. Experiments were performed to investigate the existence of a relationship between these changes and to obtain information about the mechanism involved in 1,25(OH)2D3-induced modifications in cardiac protein phosphorylation.

Dibutyryl cyclic AMP (10 microM) and forskolin (10 microM), known activators of the cAMP pathway, produced time courses of changes in <sup>45</sup>Ca uptake by chick heart tissue similar to 1,25(OH)<sub>2</sub>D<sub>3</sub> (10<sup>-10</sup> M). Analogously to the hormone, the effects of both compounds were abolished by nifedipine (30 microM) and verapamil (10 microM). In agreement with these observations, 1,25(OH)<sub>2</sub>D<sub>3</sub> significantly increased (34-70%) heart muscle cAMP levels within 1-10 min of treatment. In addition, 1,25(OH)<sub>2</sub>D<sub>3</sub> and forskolin caused similar changes in cardiac microsomal membrane protein phosphorylation (e.g. stimulation in 43 kDa and 55 kDa proteins). These changes were also evidenced by direct exposure of isolated heart microsomes to 1,25(OH)<sub>2</sub>D<sub>3</sub>, suggesting a direct membrane action of the hormone. The fast effects of 1,25(OH)<sub>2</sub>D<sub>3</sub> on dihydropyridine-sensitive cardiac muscle Ca uptake could be reproduced in primary-cultured myocytes isolated from chick embryonic heart. Furthermore, the effects of the hormone could be suppressed by a specific protein kinase A inhibitor. These results suggest that 1,25(OH)<sub>2</sub>D<sub>3</sub> affects heart cell calcium metabolism through regulation of Ca channel activity mediated by the cAMP pathway.

### **1,25(OH)<sub>2</sub> vitamin D<sub>3</sub>, and retinoic acid antagonize endothelin-stimulated hypertrophy of neonatal rat cardiac myocytes.**

Wu J; Garami M; Cheng T; Gardner DG

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J Clin Invest (United States) Apr 1 1996, 97 (7) p1577-88

1,25(OH)<sub>2</sub> Vitamin D<sub>3</sub> (VD<sub>3</sub>) and retinoic acid (RA) function as ligands for nuclear receptors which regulate transcription. Though the cardiovascular system is not thought to represent a classical target for these ligands, it is clear that both cardiac myocytes and vascular smooth muscle cells respond to these agents with changes in growth characteristics and gene expression. In this study we demonstrate that each of these ligands suppresses many of the phenotypic correlates of endothelin-induced hypertrophy in a cultured neonatal rat cardiac ventriculocyte model. Each of these agents reduced endothelin-stimulated ANP secretion in a dose-dependent fashion and the two in combination proved to be more effective than either agent used alone (VD<sub>3</sub>: 49%; RA:52%; VD<sub>3</sub> + RA:80% inhibition). RA, at concentrations known to activate the retinoid X receptor, and, to a lesser extent, VD<sub>3</sub> effected a reduction in atrial natriuretic peptide, brain natriuretic peptide, and alpha-skeletal actin mRNA levels. Similar inhibition (VD<sub>3</sub>:30%; RA:33%; VD<sub>3</sub> + RA:59% inhibition) was demonstrated when cells transfected with reporter constructs harboring the relevant promoter sequences were treated with VD<sub>3</sub> and/or RA for 48 h. These effects were not accompanied by alterations in endothelin-induced c-fos, c-jun, or c-myc gene expression, suggesting either that the inhibitory locus responsible for the reduction in the mRNA levels lies distal to the activation of the immediate early gene response or that the two are not mechanistically coupled. Both VD<sub>3</sub> and RA also reduced [<sup>3</sup>H]leucine incorporation (VD<sub>3</sub>:30%; RA:33%; VD<sub>3</sub> + RA:45% inhibition) in endothelin-stimulated ventriculocytes and, once again, the combination of the two was more effective than either agent used in isolation.

Finally, 1,25(OH)<sub>2</sub> vitamin D<sub>3</sub> abrogated the increase in cell size seen after endothelin treatment. These findings suggest that the liganded vitamin D and retinoid receptors are capable of modulating the hypertrophic process in vitro and that agents acting through these or similar signaling pathways may be of value in probing the molecular mechanisms underlying hypertrophy.

**[Effect of vitamin E deficiency on the development of cardiac arrhythmias as affected by acute ischemia]**

Belkina LM; Arkhipenko IuV; Dzhaparidze LM; Saltykova VA; Meerson FZ  
Biull Eksp Biol Med (USSR) Nov 1986, 102 (11) p530-2

Malonic dialdehyde content was increased by 53% in the myocardium of male Wistar rats (250-300 g) devoid of vitamin E for 2 months, as compared to the control rats (animals receiving an optimal amount of vitamin E). Transitory ischemia (10 min) with subsequent reoxygenation (5 min) was induced during open heart surgery under urethan anesthesia. Ischemia was induced by the occlusion of the descending branch of the left coronary artery. In ischemic rats with vitamin E deficiency the incidence of ventricular fibrillation, tachycardia, extrasystoles and the additive duration of arrhythmias were significantly increased as compared to the control.

**Antioxidant protection against adrenaline-induced arrhythmias in rats with chronic heart hypertrophy.**

Kirshenbaum LA; Gupta M; Thomas TP; Singal PK  
Division of Cardiovascular Sciences, St Boniface General Hospital Research Centre, Winnipeg, Manitoba.  
Can J Cardiol (Canada) Mar 1990, 6 (2) p71-4

Effects of vitamin E on adrenaline-induced arrhythmias were examined in rats with chronic heart hypertrophy subsequent to narrowing of the abdominal aorta. After 60 weeks of pressure overload, the rats showed an increase of about 21% in heart/body weight ratio and a small but significant rise in left ventricular end diastolic pressure (LVEDP) (sham control 1.7 +/- 0.67 mmHg; hypertrophy 7.1 +/- 2.7 mmHg) without any change in left ventricular peak systolic pressure (LVSP). Intravenous infusion of adrenaline caused rhythm disorders in a dose-dependent manner and pathological arrhythmias (occurrence of six premature ventricular complexes/min) were observed at doses of 2.9 +/- 0.6 and 3.8 +/- 1.0 micrograms/kg of the drug in control and hypertrophy animals, respectively. Administration of two doses of vitamin E (50 mg/kg intraperitoneally), given 24 h and 1 h before adrenaline infusion, significantly increased the amount of adrenaline required to produce pathological arrhythmias (control 8.0 +/- 3.0; hypertrophy 7.7 +/- 2.0 micrograms/kg). Vitamin E pretreatment did not have any detrimental effect on the pressure readings nor did it have any influence on

adrenaline-induced pressure changes. The data suggest that a combination therapy with vitamin E may allow therapeutic use of higher concentrations of adrenaline required to improve function in failing hearts with a reduced risk of arrhythmias

### **The antiarrhythmic effects of taurine alone and in combination with magnesium sulfate on ischemia/reperfusion arrhythmia**

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Chinese Pharmacological Bulletin (China), 1994, 10/5 (358-362)

The effect of taurine (Taur) alone and in combination with magnesium sulfate (MgSO<sub>4</sub>) on ischemia/reperfusion arrhythmia was investigated. The arrhythmia as produced by coronary artery occlusion for 10 min followed by reperfusion. In addition, the present study also observed the effect of MgSO<sub>4</sub> alone and in combination with Taur on hemodynamics. The results showed that Taur (50 mg . kg<sup>-1</sup>) and MgSO<sub>4</sub> (25 mg . kg<sup>-1</sup>) had partly antiarrhythmic effect. Taur (100, 150mg. kg<sup>-1</sup>) MgSO<sub>4</sub> (50, 100mg. kg<sup>-1</sup>) had significantly antiarrhythmic effect. Taur (50 mg. kg<sup>-1</sup>) combined with MgSO<sub>4</sub> (25 mg. kg<sup>-1</sup>) shortened the duration of ventricular tachycardia (VT) more than that either drug did alone. The hypotensive effect of MgSO<sub>4</sub> (25 mg. kg<sup>-1</sup>) was not increased by coadministration of Taur, but the myocardial oxygen consumption was reduced. These findings indicate that Taur in combination with MgSO<sub>4</sub> is more effect on reperfusion arrhythmia, and that the mechanism of antiarrhythmic effect of Taur and MgSO<sub>4</sub> may be involved in the effect of defence on myocardium.

### **The effects of antioxidants on reperfusion dysrhythmias**

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Ceska a Slovenska Farmacie (Czech Republic), 1995, 44/5 (257-260)

The present study aims to investigate the effects of the lipophilic antioxidant Trolox C (a vitamin E analogue) and stobadine, a scavenger of free oxygen radicals, on reperfusion dysrhythmias. Experiments were performed on isolated perfused rat hearts subjected to global stop-flow ischaemia followed by reperfusion. Trolox C (10<sup>-4</sup> mol.l<sup>-1</sup>) and stobadine (10<sup>-5</sup> mol.l<sup>-1</sup>) were infused immediately prior to ischaemia. Trolox C (10<sup>-4</sup> mol.l<sup>-1</sup>) and stobadine (10<sup>-5</sup> mol.l<sup>-1</sup>) decreased the incidence and duration of reperfusion-induced dysrhythmias (quantified by the dysrhythmia score) in comparison to the ischaemic-reperfusion damaged hearts. There was an improvement in the recovery of contraction force and left ventricular diastolic pressure in Trolox or stobadine pretreated hearts. No significant changes in coronary flow resistance were observed. The results suggest that both substances protect the myocardium

during ischaemic-reperfusion injury probably by affecting the generation and activity of reactive oxygen species.

**Protective effects of all-trans-retinoic acid against cardiac arrhythmias induced by isoproterenol, lysophosphatidylcholine or ischemia and reperfusion**

Kang JX; Leaf A

Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA.  
J Cardiovasc Pharmacol (United States) Dec 1995, 26 (6) p943-8

Previous studies have shown that free polyunsaturated fatty acids (PUFA) reduce the excitability of cardiac myocytes and exert antiarrhythmic effects. Therefore, we hypothesized that retinoic acid (RA, vitamin A acid), which has structural characteristics similar to those of PUFA, may have similar antiarrhythmic effects. To test this hypothesis, we used an isolated, spontaneously beating, neonatal rat cardiac myocyte preparation to examine the effects of RA, added to the perfusion solution, on the cell contraction and arrhythmias induced by isoproterenol (ISO) or lysophosphatidylcholine (LPC). All-trans-RA (10-20 microM) induced a marked and reversible reduction in the contraction rate of the cell in 2-5 min without changing the amplitude of the contractions. Superfusion of the myocytes with either ISO (3 microM) or LPC (5 microM) induced sustained tachyarrhythmias characterized by spasmodic contractures and fibrillation. Addition of 15-20 microM all-trans-RA to the perfusion solution effectively prevented as well as terminated the arrhythmias induced by ISO and LPC. Furthermore, in a whole-animal model of arrhythmia in which the left anterior descending coronary artery (LAD) of the anesthetized rat was occluded for 15 min followed by reperfusion, both the incidence and severity of ventricular tachycardia and fibrillation (VT, VF) were significantly reduced during the ischemic and reperfusion periods by intravenous infusion of all-trans-RA. In contrast, other analogues, including retinol and retinal, and other fat-soluble vitamins, including vitamin D, E, and K, did not have such effects. Our results demonstrate that all-trans-RA can produce antiarrhythmic effects similar to those of PUFA, suggesting a novel role of RA as a potential antiarrhythmic agent.

**Effects of dietary supplementation with alpha-tocopherol on myocardial infarct size and ventricular arrhythmias in a dog model of ischemia-reperfusion**

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Lyon, France.  
J. Am. Coll. Cardiol. (USA), 1994, 24/6 (1580-1585)

**Objectives.** We investigated whether dietary supplementation with the antioxidant vitamin alpha-tocopherol (500 mg daily) might reduce lethal ventricular arrhythmias and infarct size.

**Background.** Previous studies suggested that dietary supplementation with alpha-tocopherol may be associated with a reduced risk of ischemic heart disease. However, the mechanism of this protection remains unknown.

**Methods.** Beagle dogs were randomized to either a supplemented or a control group. Because of the low mortality rate in the supplemented group, five dogs were added to the control group. After 2 months, dogs were anesthetized and underwent a 2-h coronary artery occlusion and 6-h reperfusion. Plasma vitamin E, retinol and malondialdehyde concentrations were assessed in all dogs.

**Results.** Fourteen dogs (11 of 25 control vs. 3 of 19 supplemented dogs,  $p < 0.05$ ) developed ventricular fibrillation during either ischemia or reperfusion. Malondialdehyde concentrations were higher in dogs that subsequently developed arrhythmias (2.7 plus or minus 0.2 micromol/liter, mean plus or minus SEM) compared with dogs that did not (2.1 plus or minus 0.2 micromol/liter,  $p = 0.03$ ). Among survivors with significant ischemia, infarct size was larger in supplemented ( $n = 12$ , 58.5 plus or minus 3.3% of area at risk) than in control ( $n = 11$ , 41.9 plus or minus 6.5%,  $p < 0.04$ ) dogs. In addition, for a given collateral flow, supplemented dogs ( $n = 16$ ) developed larger infarct size than control dogs ( $n = 15$ ,  $p < 0.001$ , analysis of covariance).

**Conclusions.** The data suggest that dietary alpha-tocopherol supplementation prevented lethal ventricular arrhythmias associated with ischemia and reperfusion. However, its influence on infarct size and long-term prognosis warrants further investigation.

### **Magnesium flux during and after open heart operations in children.**

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Ann Thorac Surg (United States) Apr 1995, 59 (4) p921-7

Hypomagnesemia and depletion of the body's magnesium stores is known to be associated with an increased incidence of both cardiac arrhythmias and neurological irritability. In a two-part prospective study we have evaluated whether magnesium deficiency is a significant occurrence in children treated in the intensive care unit after open heart operations, and subsequently have sought to identify how intraoperative metabolic changes were related to the resultant findings. In 41 children studied after operation the plasma magnesium concentration showed a significant decrease from 0.92 mmol/L (10th to 90th centile, 0.71 to 1.15 mmol/L) immediately after operation to 0.77 mmol/L (0.65 to

0.91 mmol/L) on the following morning. The subsequent change in grouped values was not significant but 14 (34.2%) and 7 (17.1%) possessed values of less than 0.7 mmol/L and 0.6 mmol/L, respectively. The occurrence of cardiac arrhythmias was not statistically related to the occurrence of hypomagnesemia. In 21 children perioperative changes in extracellular and tissue magnesium, potassium, and calcium content were measured. It was found that hemodilution with a prime low in magnesium caused a reduction from a median of 0.81 mmol/L to 0.61 mmol/L ( $p < 0.01$ ). Plasma potassium level, however, was elevated from 3.7 mmol/L to 4.15 mmol/L ( $p < 0.05$ ) and the ionized calcium content from 1.17 mmol/L (1.07 to 1.25 mmol/L) to 1.49 mmol/L (1.25 to 2.56 mmol/L) ( $p = 0.0009$ ). The myocardial content of magnesium did not change significantly but skeletal muscle content was depleted from 6.75 mmol/g (2.85 to 8.35 mmol/g) to 5.65 mmol/g (2.45 to 7.2 mmol/g) ( $p < 0.01$ )

### **Sino-atrial Wenckebach conduction in thyrotoxic periodic paralysis: a case report.**

Chia BL, Lee KH, Cheah JS

Department of Medicine, National University Hospital, National University of Singapore.

Int J Cardiol (Ireland) Jan 6 1995, 47 (3) p285-9

A 28-year-old male presented with thyrotoxic periodic paralysis. On admission to hospital the serum potassium level was 1.4 mmol/l. The ECG showed classical features of hypokalaemia. In addition, sino-atrial block with Wenckebach conduction was also present. With the normalization of the serum potassium, the ECG became completely normal and showed no evidence of any arrhythmia .

### **A possible beneficial effect of selenium administration in antiarrhythmic therapy.**

Lehr D

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J Am Coll Nutr (United States) Oct 1994, 13 (5) p496-8

**OBJECTIVE:** The following review of the literature on the importance of Selenium (Se) in myocardial homeostasis and of the pharmacology of this trace metal, represents an attempt to search, without prejudice to other possible explanations, for a rationale of a beneficial effect of Se substitution as an adjuvant to antiarrhythmic therapy.

**BACKGROUND:** For several years, in the early 1980s, I had to deal with the problem of a serious ventricular arrhythmia (non-sustained and sustained ventricular tachycardia) which was remarkably resistant to a battery of the most potent antiarrhythmic agents. Eventually, dramatic improvement, lasting for a

period of 8 years, was achieved with Flecainide, which, however, left unsolved the episodic occurrence of disabling ventricular bigemini. Over the most recent period of 1 year and 8 months, there was a sudden and unexplained return to unbroken normal sinus rhythm. Among the multiplicity of possible reasons for this fortunate development, the concurrent introduction of Se substitution appeared as the most obvious, though very tentative explanation. Substitution of this trace metal preceded the extinction of ventricular bigemini by 1 week and actually represented the sole modification of otherwise reasonably standardized conditions of antiarrhythmic therapy, life style and diet. (25 Refs.)

### **Omega-3 fatty acids and prevention of ventricular fibrillation.**

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Prostaglandins Leukot Essent Fatty Acids 1995 Feb-Mar;52(2-3):197-8

Interest in the potential cardiovascular benefits of omega-3 long chain polyunsaturated fatty acids has been largely focused on possible antiatherothrombotic effects. In addition, however, definitive antiarrhythmic effects of these dietary omega-3 fatty acids have been reported by Charnock & McLennan. Our studies commenced with the observation that two of these fatty acids, eicosapentaenoic (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) prevented contracture and fibrillation of isolated neonatal cardiac myocytes when exposed to toxic levels of ouabain (0.1 mM). This protection was associated with prevention of excessively high intracellular calcium concentrations in the myocyte. Further, it was shown that these fatty acids modulate calcium currents through L-type calcium channels and that the effect occurs within a few minutes of adding EPA or DHA to the medium perfusing the cultured cardiac myocytes. Infusing an emulsion of the omega-3 fatty acids intravenously just prior to compression of a coronary artery in a conscious, prepared dog will prevent the expected subsequent ischemia-induced ventricular fibrillation. (9 Refs.)

### **An expanded concept of "insurance" supplementation--broad-spectrum protection from cardiovascular disease.**

McCarty MF

Med Hypotheses (England) Oct 1981, 7 (10) p1287-1302

The preventive merits of "nutritional insurance" supplementation can be considerably broadened if meaningful doses of nutrients such as mitochondrial "metavitamins" (coenzyme Q, lipoic acid, carnitine), lipotropes, and key essential fatty acids, are included in insurance supplements. From the standpoint of cardiovascular protection, these nutrients, as well as magnesium, selenium, and GTF-chromium, appear to have particular value. Sophisticated insurance

supplementation would likely have a favorable impact on many parameters which govern cardiovascular risk--serum lipid profiles, blood pressure, platelet stability, glucose tolerance, bioenergetics, action potential regulation--and as a life-long preventive health strategy might confer substantial benefit. (111 Refs.)

### **Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure (interim analysis)**

Baggio E, Gandini R, Plancher AC, Passeri M, Carmosino G  
Department of Internal Medicine, V. Buzzi Hospital, Milan.  
Clin Investig (Germany) 1993, 71 (8 Suppl) pS145-9

Digitalis, diuretics, and vasodilators are considered standard therapy for patients with congestive heart failure, for which treatment is tailored according to the severity of the syndrome and the patient profile. Apart from the clinical seriousness, heart failure is always characterized by an energy depletion status, as indicated by low intramyocardial ATP and coenzyme Q10 levels. We investigated safety and clinical efficacy of coenzyme Q10 (CoQ10) adjunctive treatment in congestive heart failure, which had been diagnosed at least 6 months previously and treated with standard therapy. A total of 2500 patients in NYHA classes II and III were enrolled in this open noncomparative 3-month postmarketing drug surveillance study in 173 Italian centers. The daily dose of CoQ10 was 50-150 mg orally, with the majority of patients (78%) receiving 100 mg/day. Clinical and laboratory parameters were evaluated at the entry into the study and on day 90; the assessment of clinical signs and symptoms was made using from two- to seven-point scales. Preliminary results on 1113 patients (mean age 69.5 years) show a low incidence of side effects: 10 adverse reactions were reported in 8 (0.8%) patients, of which only 5 reactions were considered as correlated to the test treatment. After 3 months of test treatment the proportions of patients with improvement in clinical signs and symptoms were as follows: cyanosis 81%, edema 76.9%, pulmonary rales 78.4%, enlargement of the liver area 49.3%, jugular reflux 81.5%, dyspnea 54.2%, palpitations 75.7%, sweating 82.4%, arrhythmia 62%, insomnia 60.2%, vertigo 73%, and nocturia 50.7%.

### **Isolated diastolic dysfunction of the myocardium and its response to CoQ10 treatment.**

Langsjoen PH, Langsjoen PH, Folkers K  
Clin Investig (Germany) 1993, 71 (8 Suppl) pS140-4

Symptoms of fatigue and activity impairment, atypical precordial pain, and cardiac arrhythmia frequently precede by years the development of congestive heart failure. Of 115 patients with these symptoms, 60 were diagnosed as having hypertensive cardiovascular disease, 27 mitral valve prolapse syndrome, and 28 chronic fatigue syndrome. These symptoms are common with diastolic

dysfunction, and diastolic function is energy dependent. All patients had blood pressure, clinical status, coenzyme Q10 (CoQ10) blood levels and echocardiographic measurement of diastolic function, systolic function, and myocardial thickness recorded before and after CoQ10 replacement. At control, 63 patients were functional class III and 54 class II; all showed diastolic dysfunction; the mean CoQ10 blood level was 0.855 micrograms/ml; 65%, 15%, and 7% showed significant myocardial hypertrophy, and 87%, 30%, and 11% had elevated blood pressure readings in hypertensive disease, mitral valve prolapse and chronic fatigue syndrome respectively. Except for higher blood pressure levels and more myocardial thickening in the hypertensive patients, there was little difference between the three groups. CoQ10 administration resulted in improvement in all; reduction in high blood pressure in 80%, and improvement in diastolic function in all patients with follow-up echocardiograms to date; a reduction in myocardial thickness in 53% of hypertensives and 36% of the combined prolapse and fatigue syndrome groups; and a reduced fractional shortening in those high at control and an increase in those initially low.(ABSTRACT TRUNCATED AT 250 WORDS)

### **Protective effects of propionyl-L-carnitine during ischemia and reperfusion.**

Shug A, Paulson D, Subramanian R, Regitz V  
University of Wisconsin Medical School, Madison.  
Cardiovasc Drugs Ther (United States) Feb 1991, 5 Suppl 1 p77-83

When cardiac function in isolated rat hearts was impaired by subjecting them to ischemia, subsequent perfusion with propionyl-L-carnitine and related compounds increased their rate of recovery. Thus at 11 mM, both propionyl-L-carnitine and, to a lesser extent, its taurine amide, and also acetyl-L-carnitine, significantly restored cardiac function in 15 minutes after 90 minutes of either low-flow or intermittent no-flow ischemia. Carnitine itself was ineffective. Propionyl-L-carnitine also increased tissue ATP and creatine phosphate compared with controls, but did not affect the levels of long-chain acyl carnitine and coenzyme. These esters also depleted fatty acid peroxidation, as shown with malonaldehyde, and were more effective than carnitine in preventing the production of superoxide. In myocytes, propionyl-L-carnitine alone stimulated palmitate oxidation, but in rat heart homogenates, both L-carnitine and propionyl-L-carnitine did so, while acetyl-L-carnitine was actually inhibitory. Possible mechanisms for the protective action of propionyl-L-carnitine against ischemia include an increased rate of cellular transport, stimulation of fatty acid oxidation, and a reduction of free radical formation.

### **Community-based prevention of stroke: nutritional improvement in Japan**

Yamori Y, Horie R  
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Health Rep 1994;6(1):181-8

**OBJECTIVES:** (1) To demonstrate the importance of nutrition, especially sodium restriction and increased potassium and protein intakes, in the prevention of hypertension and stroke in a pilot study involving senior citizens. (2) To design a population-based intervention in the Shimane Prefecture of Japan concerning dietary factors such as low sodium and high potassium, protein, magnesium, calcium and dietary fibre in the prevention of stroke.

**DESIGN AND METHODS:** The intervention study was carried out at a senior citizens' residence and included general health education along with a reduction of dietary salt intake and increases in vegetable and protein, especially from seafood. Sixty-three healthy senior citizens (average age: 74.8 +/- 7.7 years) had their daily meals modified to a low sodium/potassium ratio for four weeks without their knowledge by the use of a potassium chloride substitute for salt, soy sauce and bean paste, which contains much less sodium and more potassium. Monosodium L-glutamate monohydrate used for cooking was changed to monopotassium L-glutamate monohydrate. Blood pressure was measured with the patient in the sitting position. Daily dietary sodium and potassium intakes were assessed by flame photometry from 24-hour urine specimens. Extensive intervention programs were introduced into the Shimane Prefecture, which has a population of 750,000, through health education classes for housewives, home visits by health nurses and an educational TV program for dietary improvement. The mortality from stroke was monitored for 10 years and compared with the average in Japan.

**RESULTS:** The blood pressure lowering effect of reducing the dietary sodium/potassium ratio was confirmed through a pilot intervention study at the senior citizens' residence. The mortality rates for stroke in the middle-aged population from the Shimane Prefecture during the 10 years after the introduction of dietary improvement had a steeper decline in hemorrhagic, ischemic and all strokes than the average for Japan.

### **Effect of dietary magnesium supplementation on intralymphocytic free calcium and magnesium in stroke-prone spontaneously hypertensive rats.**

Adachi M; Nara Y; Mano M; Yamori Y

Department of Pathology, Shimane Medical University, Izumo, Japan.

Clin Exp Hypertens 1994 May;16(3):317-26

The effects of dietary magnesium (Mg) supplementation on intralymphocytic free  $Ca^{2+}$  ( $[Ca^{2+}]_i$ ) and  $Mg^{2+}$  ( $[Mg^{2+}]_i$ ) were examined in the stroke-prone spontaneously hypertensive rats (SHRSP) at the age of 10 weeks. After 40 day Mg supplementation (0.8% Mg in the diet), systolic blood pressure (SBP) was significantly lower in Mg supplemented group (Mg group) than the control group (0.2% Mg).  $[Ca^{2+}]_i$  was significantly lower and  $[Mg^{2+}]_i$  was significantly higher in Mg group than in the control group. Further,  $[Ca^{2+}]_i$  was positively and  $[Mg^{2+}]_i$  was negatively correlated with SBP. These results suggest that dietary Mg supplementation modifies  $[Ca^{2+}]_i$  and  $[Mg^{2+}]_i$ , and modulates the development of hypertension.

**Clinical study of cardiac arrhythmias using a 24-hour continuous electrocardiographic recorder (5th report)--antiarrhythmic action of coenzyme Q10 in diabetics.**

Fujioka T, Sakamoto Y, Mimura G  
Tohoku J Exp Med (Japan) Dec 1983, 141 Suppl p453-63

An investigation was undertaken to evaluate the antiarrhythmic effect of CoQ10 on VPBs using the Holter ECG, in 27 patients with no clinical findings of organic cardiopathies. As a result, the effect of CoQ10 on VPBs was considered beneficial in 6 (22%) of 27 cases, consisting of 1 patient with hypertension and 5 patients with DM. Even in the remaining 2 patients with DM, the frequency of VPBs was reduced by 50% or more during treatment with CoQ10. The mean reduction of VPBs frequency in the 5 responders plus these 2 patients with DM was 85.7%. These findings suggest that CoQ10 exhibits an effective antiarrhythmic action not merely on organic heart disease but also on VPBs supervening on DM.

**Usefulness of coenzyme Q10 in clinical cardiology: a long-term study.**

Langsjoen H, Langsjoen P, Langsjoen P, Willis R, Folkers K  
University of Texas Medical Branch, Galveston 77551, USA.  
Mol Aspects Med 1994;15 Suppl:s165-75

Over an eight year period (1985-1993), we treated 424 patients with various forms of cardiovascular disease by adding coenzyme Q10 (CoQ10) to their medical regimens. Doses of CoQ10 ranged from 75 to 600 mg/day by mouth (average 242 mg). Treatment was primarily guided by the patient's clinical response. In many instances, CoQ10 levels were employed with the aim of producing a whole blood level greater than or equal to 2.10 micrograms/ml (average 2.92 micrograms/ml, n = 297). Patients were followed for an average of 17.8 months, with a total accumulation of 632 patient years. Eleven patients were omitted from this study: 10 due to non-compliance and one who experienced nausea. Eighteen deaths occurred during the study period with 10 attributable to cardiac causes. Patients were divided into six diagnostic categories: ischemic cardiomyopathy (ICM), dilated cardiomyopathy (DCM), primary diastolic dysfunction (PDD), hypertension (HTN), mitral valve prolapse (MVP) and valvular heart disease (VHD). For the entire group and for each diagnostic category, we evaluated clinical response according to the New York Heart Association (NYHA) functional scale, and found significant improvement. Of 424 patients, 58 per cent improved by one NYHA class, 28% by two classes and 1.2% by three classes. A statistically significant improvement in myocardial function was documented using the following echocardiographic parameters: left ventricular wall thickness, mitral valve inflow slope and fractional shortening. Before treatment with CoQ10, most patients were taking from one to five cardiac medications. During this study, overall medication requirements dropped

considerably: 43% stopped between one and three drugs. Only 6% of the patients required the addition of one drug. No apparent side effects from CoQ10 treatment were noted other than a single case of transient nausea. In conclusion, CoQ10 is a safe and effective adjunctive treatment for a broad range of cardiovascular diseases, producing gratifying clinical responses while easing the medical and financial burden of multidrug therapy.

### **Effect of coenzyme Q10 on structural alterations in the renal membrane of stroke-prone spontaneously hypertensive rats.**

Okamoto H, Kawaguchi H, Togashi H, Minami M, Saito H, Yasuda H  
Department of Cardiovascular, Hokkaido University, Japan.  
Biochem Med Metab Biol 1991 Apr;45(2):216-26

To test the hypothesis that structural abnormalities exist in the kidney membrane of spontaneously hypertensive rats, we examined the effect of long-term administration of coenzyme Q10 on membrane lipid alterations in the kidney of stroke-prone spontaneously hypertensive rats (SHRSP). As compared with normotensive Wistar-Kyoto rats, renal membrane phospholipids, especially phosphatidylcholine and phosphatidylethanolamine, decreased and renal phospholipase A2 activity was enhanced with age in untreated SHRSP. Treatment with coenzyme Q10 attenuated the elevation of blood pressure, the membranous phospholipid degradation, and the enhanced phospholipase A2 activity. These results suggest that one factor contributing to the progress of hypertension is a structural membrane abnormality that alters the physical and functional properties of the cell membrane, and coenzyme Q10 might protect the renal membrane from damage due to hypertension in SHRSP.

### **Co-enzyme Q10: a new drug for cardiovascular disease.**

Greenberg S, Frishman WH  
Department of Medicine, Mt. Sinai Hospital and Medical Center, New York, New York.  
J Clin Pharmacol 1990 Jul;30(7):596-608

Co-enzyme Q10 (ubiquinone) is a naturally occurring substance which has properties potentially beneficial for preventing cellular damage during myocardial ischemia and reperfusion. It plays a role in oxidative phosphorylation and has membrane stabilizing activity. The substance has been used in oral form to treat various cardiovascular disorders including angina pectoris, hypertension, and congestive heart failure. Its clinical importance is now being established in clinical trials worldwide.

**[Effects of 2,3-dimethoxy-5-methyl-6-(10'-hydroxydecyl)-1,4-benzoquinone (CV-2619) on adriamycin-induced ECG abnormalities and myocardial energy metabolism in spontaneously hypertensive rats]**

Shimamoto N, Tanabe M, Hirata M  
Nippon Yakurigaku Zasshi 1982 Oct;80(4):307-15

Antidote actions of CV-2619 and ubiquinone-10 (Q-10) against adriamycin (ADM) cardiotoxicity were studied in spontaneously hypertensive rats. ADM (1 mg/kg/day, i.p.) elicited widening of the QRS complex in the ECG. The widening of the QRS complex was counteracted by a 10-day treatment with CV-2619 (10 and 30 mg/kg/day, p.o.) or Q-10 (10 mg/kg/day, p.o.), which was started on the 15th day of the ADM treatment. CV-2619 or Q-10, however, did not influence ADM-induced decrease in body and heart ventricular weights. Systemic hypotension caused by adriamycin was accelerated by CV-2619 or Q-10. The ADM treatment significantly decreased myocardial glycogen and glucose contents, while it did not affect the lactate content. Furthermore, ADM did not affect the myocardial content of adenine nucleotides, but significantly increased that of creatine phosphate. CV-2619 or Q-10 medication did not counteract changes in these contents by ADM. On the contrary, both agents decreased the lactate content and increased the phosphorylation potential, an index of myocardial energy state. In conclusion, CV-2619 might be as effective as Q-10 to protect the heart against ADM cardiotoxicity, and both test agents improved the myocardial energy state.

**Bioenergetics in clinical medicine. III. Inhibition of coenzyme Q10-enzymes by clinically used anti-hypertensive drugs.**

Kishi H, Kishi T, Folkers K  
Res Commun Chem Pathol Pharmacol 1975 Nov;12(3):533-40

Background data revealed that some American and Japanese patients with essential hypertension, including many who were not being treated with any anti-hypertensive drug, had a deficiency of coenzyme Q10. Eight clinically used anti-hypertensive drugs have now been tested for inhibition of two mitochondrial coenzyme Q10-enzymes of heart tissue, succinoxidase and NADH-oxidase. Diazoxide and propranolol significantly inhibited the CoQ10-succinoxidase and CoQ10-NADH-oxidase, respectively. Metoprolol did not inhibit succinoxidase, and was one-fourth as active as propranolol for inhibition of NADH-oxidase. Hydrochlorothiazide, hydralazine, and clonidine also inhibited CoQ10-NADH-oxidase. Reserpine did not inhibit either CoQ10-enzyme, and methyldopa was a very weak inhibitor of succinoxidase. The internationally recognized clinical side-effects of propranolol may be due, in part, to inhibition of CoQ10-enzymes which are indispensable in the bioenergetics of cardiac function. A pre-existing deficiency of coenzyme Q10 in the myocardium of hypertensive patients could be augmented by subsequent treatment with propranolol, possibly to the "life-threatening" state described by others.

## **Bioenergetics in clinical medicine. Studies on coenzyme Q10 and essential hypertension.**

Yamagami T, Shibata N, Folkers K

Res Commun Chem Pathol Pharmacol 1975 Jun;11(2):273-88

The specific activities (S.A.) of the succinate dehydrogenase-coenzyme Q10 (CoQ10) reductase of a control group of 65 Japanese adults and 59 patients having essential hypertension were determined. The mean S.A. of the hypertensive group was significantly lower ( $p$  less than 0.001) and the mean % deficiency of enzyme activity was significantly higher ( $p$  less than 0.001) than the values for the control group. These data on Japanese in Osaka agree with data on Americans in Dallas. Some patients showed no CoQ10-deficiency, and others showed definite deficiencies. Emphasizing the CoQ10-enzyme for patient selection, CoQ10 was administered to hypertensive patients. Four individuals showed significant but partial reductions of blood pressure. Monitoring the CoQ10-enzyme before, during, and after administration of CoQ10 indicated responses. The maintenance of high blood pressure could be primarily due to contraction of the arterial wall. Contraction or relaxation of an arterial wall is dependent upon bioenergetics, which also provide the energy for biosynthesis of angiotensin II, renin, aldosterone, and the energy for sodium and potassium transport. A clinical benefit from administration of CoQ10 to patients with essential hypertension could be based upon correcting a deficiency in bioenergetics, and point to possible combination treatments with a form of CoQ and anti-hypertensive drugs.

## **[Prevention of cerebrovascular insults]**

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Schweiz Med Wochenschr 1994 Nov 12;124(45):1995-2004

Cerebrovascular infarction is the third leading cause of mortality following coronary heart disease and malignancies. WHO studies show that more than half of patients admitted for cerebrovascular infarction were not treated for hypertension. The risk factors for coronary heart disease and cerebrovascular infarction are not identical. Patients with systolic and diastolic hypertension, atrial fibrillation, stenosis of the carotid artery, and smoking, have a significantly elevated risk for cerebrovascular accidents. Hypercholesterolemia and diabetes are less important risk factors. Risk factors amendable by adequate nutritional intake are low supply of carotene and vitamin C. Homocysteineemia appears to be a risk factor that may be influenced by appropriate nutrition. Antihypertensive therapy is the most important primary and secondary preventive measure. No smoking and adequate dietary intake are also important. Primary prevention with low dose salicylic acid (ASA) is recommended in the presence of additional

cardiovascular risk factors. The benefit of low dose anticoagulant therapy in atrial fibrillation without symptoms is not fully established. In subjects with atrial fibrillation with cerebrovascular events anticoagulants are superior to ASA. Surgical treatment of significant stenosis of the carotid artery is indicated. In secondary prevention of thromboembolic events, low dose ASA is recommended. A valuable alternative in case of side effects is available in ticlopidine.

### **[Essential antioxidants in cardiovascular diseases--lessons for Europe]**

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Ther Umsch 1994 Jul;51(7):475-82

Complementary epidemiological studies consistently reveal a substantially increased risk of cardiovascular disease (CVD) at suboptimal plasma levels of essential antioxidants in comparison with optimum ranges of vitamin C (> 50  $\mu\text{mol/l}$ ), of lipid-standardized vitamin E (> 30  $\mu\text{mol/l}$  or a tocopherol/cholesterol ratio > 5.2  $\mu\text{mol/mmol}$ ), beta-carotene (> 0.4  $\mu\text{mol/l}$ ). The poor level of any single essential antioxidant can increase the risk, and the combination of suboptimal levels has additive or even overmultiplicative effects on the risk for CVD. Suboptimal antioxidant levels are stronger predictors of the severalfold regional differences of CVD in Europe than classical risk factor such as hypercholesterolemia, hypertension, etc. Scotsmen and Fins tend to suboptimal levels of essential antioxidants, whereas German-speaking regions may mostly reveal a fair vitamin E status, but at least one out of four subjects can reveal suboptimal levels of vitamin C and carotene, particularly in smokers. This deficit can be avoided by 'prudent diets' rich in fruits and vegetables as practiced by Frenchmen, Italians and Spaniards. The simultaneous correction of all suboptimal antioxidant levels appears to be a promising new means for CVD prevention, particularly in the northern parts of Europe. In the USA the risk of CVD could substantially be reduced without dietary modifications by voluntary daily supplements as follows: vitamin C > 140 mg, vitamin E > 100 IU (100 mg d,l- or 74 mg d-alpha-tocopherylacetate), and in current smokers by gamma-carotene > 8.6 mg. Hence, these antioxidants may be crucial constituents of diets rich in fruits and vegetables, which are by consensus associated with a lower risk of premature death from CVD (and cancer as well).

### **Antioxidant vitamin intake and coronary mortality in a longitudinal population study.**

Knekt P, Reunanen A, Jarvinen R, Seppanen R, Heliovaara M, Aromaa A  
Social Insurance Institution, Helsinki, Finland.  
Am J Epidemiol 1994 Jun 15;139(12):1180-9

Oxidation of lipoproteins is hypothesized to promote atherosclerosis and, thus, a high intake of antioxidant nutrients may protect against coronary heart disease. The relation between the intakes of dietary carotene, vitamin C, and vitamin E and the subsequent coronary mortality was studied in a cohort of 5,133 Finnish men and women aged 30-69 years and initially free from heart disease. Food consumption was estimated by the dietary history method covering the total habitual diet during the previous year. Altogether, 244 new fatal coronary heart disease cases occurred during a mean follow-up of 14 years beginning in 1966-1972. An inverse association was observed between dietary vitamin E intake and coronary mortality in both men and women with relative risks of 0.68 (p for trend = 0.01) and 0.35 (p for trend < 0.01), respectively, between the highest and lowest tertiles of the intake. Similar associations were observed for the dietary intake of vitamin C and carotenoids among women and for the intake of important food sources of these micronutrients, i.e., of vegetables and fruits, among both men and women. The associations were not attributable to confounding by major nondietary risk factors of coronary heart disease, i.e., age, smoking, serum cholesterol, hypertension, or relative weight. The results support the hypothesis that antioxidant vitamins protect against coronary heart disease, but it cannot be excluded that foods rich in these micronutrients also contain other constituents that provide the protection.

### **The decline in stroke mortality. An epidemiologic perspective.**

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Ann Epidemiol 1993 Sep;3(5):571-5

The evidence that treatment of hypertension prevents stroke is incontrovertible. Several observations, however, suggest that improvements in the prevalence of antihypertensive treatment cannot explain all of the recent decline in stroke mortality. Changes in nutritional patterns may explain some of the observed decline. Prospective studies have demonstrated conclusively an independent, increasing risk of hemorrhagic, but not thrombotic, stroke at higher levels of alcohol use. Stroke mortality is associated inversely with fat and protein intake. Dietary sodium has been linked to stroke in ecologic studies but not in prospective studies. Ecologic studies have suggested that foods high in vitamin C and potassium protect against stroke; an inverse association of potassium intake with fatal stroke has been demonstrated in cohort studies. Two studies in humans also suggest a protective effect of serum selenium against subsequent stroke. Determination of the influence of nutrients on stroke incidence offers tantalizing opportunities for future research and possibly, intervention.

### **Can antioxidants prevent ischemic heart disease?**

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J Clin Pharm Ther 1993 Apr;18(2):85-95

Ischemic heart disease remains a major cause of mortality in developed countries. A number of important risk factors for the development of coronary atherosclerosis have been identified including hypertension, hypercholesterolaemia, insulin resistance and smoking. However, these factors can only partly explain variations in the incidence of ischaemic heart disease either between populations or within populations over time. In addition, population interventions based upon these factors have had little impact in the primary prevention of heart disease. Recent evidence suggests that one of the important mechanisms predisposing to the development of atherosclerosis is oxidation of the cholesterol-rich low-density lipoprotein particle. This modification accelerates its uptake into macrophages, thereby leading to the formation of the cholesterol-laden 'foam cell'. In vitro, low-density lipoprotein oxidation can be prevented by naturally occurring antioxidants such as vitamin C, vitamin E and beta-carotene. This article explores the evidence that these dietary anti-oxidants may influence the rate of progression of coronary atherosclerosis in vivo and discusses the need for formal clinical trials of antioxidant therapy.

#### **Antioxidant therapy in the aging process.**

Deucher GP  
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EXS 1992;62:428-37

A total of 1,265 patients with age-related diseases such as diabetes, arthritis, vascular disease and hypertension as well as 1,100 persons in diminished health without apparent disease, were treated with the metal chelator EDTA and antioxidants such as vitamin C, E, beta-carotene, selenium, zinc and chromium. Good results were observed in the majority of patients. This is encouraging for the initiation of controlled clinical trials.

## 7. Atherosclerosis

Preventative and curative options include:

- Vitamin E, vitamin C, folic acid, B vitamins, zinc, selenium, coenzyme Q10, green tea, ginkgo biloba, ginseng, bilberry, grape seed-skin, vitamin B12, vitamin B6, trimethylglycine (TMG), omega 3 fatty acids, garlic, chromium, copper, artichoke extract, niacin, ginger, curcumin, soy protein, pectins, guar, psyllium, taurine, DHEA.

### **The role of homocysteine, folate and other B-vitamins in the development of atherosclerosis.**

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Arch Latinoam Nutr (Venezuela) Jun 1997, 47 (2 Suppl 1) p9-12

Recently, elevated homocysteine blood concentrations have been identified as an independent risk factor for the development of atherosclerotic lesions. The amino acid homocysteine is metabolized in the human body involving the vitamins folic acid, B12 and B6 as essential cofactors and coenzymes, respectively. There is an inverse relationship between the status of the relevant B-vitamins and the homocysteine blood concentration. Supplementation of these vitamins results in a significant reduction of the homocysteine level. Nutritive amounts seem to be sufficient to obtain this reduction, even in the case of elevated homocysteine levels. (18 Refs.)

### **Erythrocyte selenium-glutathione peroxidase activity is lower in patients with coronary atherosclerosis.**

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Jpn Heart J (Japan) Nov 1997, 38 (6) p793-8

To obtain further insight into the role of erythrocyte antioxidant systems in the development of atherosclerosis, intraerythrocyte enzyme activities and selenium levels in erythrocytes were determined in 37 patients with angiographically proved coronary artery stenosis and 15 subjects with normal coronary angiograms as controls. In a preliminary study, the enzymatic activities of glucose-6-phosphate dehydrogenase (G6PD), glutathione reductase (GR) and selenium-dependent glutathione peroxidase (Se-GPx) were measured in both venous and arterial blood samples obtained from patients before angiography. The data of the preliminary study, which showed that only the Se-GPx decreased in the patients, led us to concentrate on the Se-GPx and Se levels to determine the changes in these variables. Our results showed that there was a decrease in both the activity of Se-GPx and Se levels in erythrocytes parallel to the increase in the severity of

coronary artery disease. It was concluded that these parameters might be used as determinants in the assessment of the severity of the disease.

**Insulin sensitivity and intake of vitamins E and C in African American, Hispanic, and non-Hispanic white men and women: the Insulin Resistance and Atherosclerosis Study (IRAS).**

Sanchez-Lugo L; Mayer-Davis EJ; Howard G; Selby JV; Ayad MF; Rewers M; Haffner S

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Am J Clin Nutr (United States) Nov 1997, 66 (5) p1224-31

Elevated fasting insulin concentrations and insulin resistance have been associated with non-insulin-dependent diabetes mellitus (NIDDM), obesity, atherosclerosis, and hypertension. Vitamin E supplementation in persons with and without NIDDM may be related to greater insulin sensitivity (SI). The cross-sectional associations of the intake of vitamins E and C with SI and insulin concentrations were evaluated among African American, Hispanic, and non-Hispanic white men and women with a wide spectrum of glucose tolerance included in the Insulin Resistance and Atherosclerosis Study (IRAS) (n = 1151). Insulin sensitivity was measured by minimal model analysis of a 12-sample, insulin-modified, frequently sampled intravenous glucose tolerance test. Nutrient intake (including vitamin supplement use) was assessed with a food-frequency questionnaire modified to include foods consumed by the three ethnic groups. Linear-regression models were used, including rank of SI and the log of fasting insulin as the outcome variables. Pearson correlation coefficients for vitamins E and C in relation to rank SI were  $r = 0.07$  ( $P = 0.01$ ) and  $r = 0.07$  ( $P = 0.02$ ), respectively. After adjustment for total energy and BMI these associations were no longer statistically significant and did not differ between ethnic groups. Results were similar when vitamins E and C were combined in categories of low and high antioxidant intake. Models replicated with log of fasting insulin as the outcome variable also did not produce significant associations with vitamins E or C. Thus, these cross-sectional analyses do not support the hypothesis of improved SI with increased intake of vitamins E and C.

**Effects of malnutrition and atherosclerosis on the fatty acid composition of plasma phospholipids in the elderly.**

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Ann Nutr Metab (Switzerland) 1997, 41 (3) p166-72

The fatty acid profiles of plasma phospholipids have been compared in 53 elderly subjects suffering from malnutrition (group U, 17 subjects) or from atherosclerosis (group A, 15 subjects). A control group was also included in the

study (group C, 21 subjects). Main differences were observed in phosphatidylcholine (PC). In group U, the proportion of monounsaturated fatty acids increased in PC, which was reflected by an increase in unsaturated fatty acids without significant modification of essential fatty acids. In group A, no major modification has been observed statistically, although the proportion of saturated fatty acids tended to increase.

### **The effect of dietary fat, antioxidants, and pro-oxidants on blood lipids, lipoproteins, and atherosclerosis**

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Journal of the American Dietetic Association (USA), 1997, 97/7 Suppl. (S31-S41)

A number of primary and secondary prevention trials, including angiographic studies, have indicated that a decrease in dietary saturated fat and cholesterol produces a decrease in the blood levels of cholesterol and low-density lipoprotein (LDL) cholesterol, leading to a decrease in coronary artery disease (CAD). Increasing evidence indicates that the oxidation of LDL in human beings is atherogenic. Of the three major antioxidants, vitamin E, beta carotene, and vitamin C, the evidence is strongest that vitamin E (at a minimum dose of 100 IU/day) has a strong and independent inverse association with CAD. Selenium and flavonoids also have antioxidant properties, but their association with CAD in human beings is equivocal. Two prooxidants, homocysteine and iron, have been found to be associated with CAD. Blood homocysteine levels can be lowered significantly by an increase in dietary folic acid. Clinical trials are needed to assess expeditiously the effect of antioxidants, particularly vitamin E, and of folic acid on CAD and atherosclerosis. The substitution of monounsaturated fat for saturated fat lowers LDL and makes it less susceptible to oxidation without decreasing high-density lipoprotein (HDL) cholesterol. Studies in transgenic mice indicate that apolipoprotein A-I, the major protein of HDL, may inhibit the oxidation of LDL. Dietary trans fatty acids at the level consumed by many Americans can increase LDL cholesterol and may decrease HDL cholesterol. Individuals who have CAD or have family members who have premature CAD have delayed clearance of dietary fat, as judged by studies of postprandial triglyceride metabolism. The importance of decreasing dietary saturated fat and cholesterol is well established, but a number of other factors appear to influence the risk of CAD significantly and provide important areas nutrition for future investigation to improve prevention and treatment through better .

### **Role of the natural antioxidants in the prevention of atherosclerosis**

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Spain  
Farmacia Clinica (Spain), 1997, 14/1 (43-48)

Many chronic pathologies, including atherosclerotic disease, are connected physiopathologically with an increase in oxidative activity. Various studies have suggested that oxidative modification of the low density lipoproteins (LDL) is fundamental in atherogenesis. If we accept that some human diseases are associated with an imbalance between oxidative stress and antioxidant defence, it is possible, at least in theory, to limit this damage and retard development of the disease by supplementing the antioxidant mechanisms. Possible therapeutic interventions may include natural antioxidants or synthetic pharmacological agents. In this article we review the scientific evidence supporting the oxidative hypothesis of atherosclerosis and examine the results of the use of dietetic antioxidants to prevent and/or retard the atherosclerotic process.

### **Antioxidant content in low density lipoprotein and lipoprotein oxidation in vivo and in vitro**

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Free Radical Research (United Kingdom), 1998, 29/2 (165-173)

Human blood contains naturally occurring multiple-modified low density Lipoprotein (nomLDL) capable of inducing the accumulation of cholesteryl esters in the cells of human aortic intima. NomLDL is desialylated particles of small size with an increased electronegative charge which can be separated from native low density lipoprotein (LDL) by lectin chromatography. The purpose of this study was to determine the content of antioxidants in native and nomLDL obtained from healthy subjects and from patients with coronary heart disease as well as to elucidate a possible relationship between the level of antioxidants and the degree of in vivo and in vitro LDL oxidizability. The apoB-bound cholesterol level in native and nomLDL of healthy subjects was 0.25 plus or minus 0.08 and 0.28 plus or minus 0.05 mol/mol apoB, respectively. The level of apoB-bound cholesterol in native LDL of coronary atherosclerosis patients showed no significant difference from that in healthy subjects' native lipoprotein. At the same time, the level of apoB-bound cholesterol in patients' nomLDL was 7-fold higher than in native LDL. The average duration of the lag phase of native LDL oxidation did not show a significant difference between the lipoprotein of healthy subjects and coronary atherosclerosis patients. The lag phase of nomLDL obtained from healthy subjects and patients was significantly shorter (3- and 6-fold, respectively) than for their native LDL. The latter finding points to their increased susceptibility to in vitro oxidation. Oxidizability of total LDL preparations correlated positively with their nomLDL content. The content of all the antioxidants studied (coenzyme-Q10, alpha- and gamma-tocopherols, beta-carotene and lycopene) in nomLDL was 1.5- to 2-fold lower than in native LDL.

The level of apoB-bound cholesterol in nomLDL, correlated positively with the ubiquinone-10 content and showed negative correlation with ubiquinol-10 and beta-carotene levels. On the other hand, the content of apoB-bound cholesterol in native LDL correlated positively with the ubiquinol-10 level. Susceptibility of nomLDL to in vitro oxidation exhibited negative correlation with alpha-tocopherol and beta-carotene levels and a positive correlation with the ubiquinone-10 content. On the contrary, oxidizability of native LDL correlated positively with the ubiquinone-10 level. Conclusions: (a) elevated apoB-bound cholesterol level in nomLDL of coronary atherosclerosis patients indicates that peroxidation of lipids occurs in vivo; (b) in vivo lipoperoxidation in nomLDL is corroborated by increased proportion of oxidized form of coenzyme-Q10; (c) content of lipid-soluble antioxidants in nomLDL is lower than in native lipoprotein; (d) nomLDL has a higher susceptibility to in vitro oxidation than native LDL; (e) it is necessary to use isolated subfractions of native LDL and nomLDL, but not total lipoprotein preparations, to study the mechanisms of lipid peroxidation.

### **Are there protective environmental factors?**

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Archives des Maladies du Coeur et des Vaisseaux (France), 1998, 91/Spec. Iss. 5  
(27-31)

Protective factors against atherosclerosis are a group of different elements which include the fatty acids, alcohol, antioxidant vitamins, dietary fibres and physical exercise. Unsaturated fatty acids, especially alpha-linolenic acid have a beneficial effect on the coronary vessels. Alpha-linolenic acid is associated with low coronary mortality both in cohort studies (the Seven Countries Study) and in secondary prevention (Lyon Diet Heart Study). There is an inverse relationship between moderate alcohol consumption and coronary artery disease with a reduction of risk of about 30% in all prospective studies. High dietary intake of vitamin E was found to be associated with a decreased coronary risk. On the other hand, dietary supplements of vitamin E in primary and secondary prevention were associated with increased cardiovascular mortality. Foliates have a protective effect by their action on homocysteine metabolism. There is no formal proof at present in favour of the systematic introduction of the B vitamins in primary or secondary prevention. Fresh fruit and vegetables seem to be protective by their fibre and vitamin B content. Moderate endurance physical exercise is a protective factor in all studies. Its beneficial effects in function and rehabilitation are well documented. In primary prevention studies, exercise has a beneficial effect but criteria of duration and frequency remain vague. Therefore, there are environmental protective factors against atherosclerosis which allow physicians to introduce a positive note in these recommendations.

## **Functional food science and the cardiovascular system**

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British Journal of Nutrition (United Kingdom), 1998, 80/Suppl. 1 (S113-S146)

Cardiovascular disease has a multifactorial aetiology, as is illustrated by the existence of numerous risk indicators, many of which can be influenced by dietary means. It should be recalled, however, that only after a cause-and-effect relationship has been established between the disease and a given risk indicator (called a risk factor in that case), can modifying this factor be expected to affect disease morbidity and mortality. In this paper, effects of diet on cardiovascular risk are reviewed, with special emphasis on modification of the plasma lipoprotein profile and of hypertension. In addition, dietary influences on arterial thrombotic processes, immunological interactions, insulin resistance and hyperhomocysteinaemia are discussed. Dietary lipids are able to affect lipoprotein metabolism in a significant way, thereby modifying the risk of cardiovascular disease. However, more research is required concerning the possible interactions between the various dietary fatty acids, and between fatty acids and dietary cholesterol. In addition, more studies are needed with respect to the possible importance of the postprandial state. Although in the aetiology of hypertension the genetic component is definitely stronger than environmental factors, some benefit in terms of the development and coronary complications of atherosclerosis in hypertensive patients can be expected from fatty acids such as alpha-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid. This particularly holds for those subjects where the hypertensive mechanism involves the formation of thromboxane A<sub>2</sub> and/or alpha<sub>1</sub>-adrenergic activities. However, large-scale trials are required to test this contention. Certain aspects of blood platelet function, blood coagulability, and fibrinolytic activity are associated with cardiovascular risk, but causality has been insufficiently proven. Nonetheless, well-designed intervention studies should be initiated to further evaluate such promising dietary components as the various n-3 and n-6 fatty acids and their combination, antioxidants, fibre, etc. for their effect on processes participating in arterial thrombus formation. Long-chain polyenes of the n-3 family and antioxidants can modify the activity of immunocompetent cells, but we are at an early stage of examining the role of immune function on the development of atherosclerotic plaques. Actually, there is little, if any, evidence that dietary modulation of immune system responses of cells participating in atherogenesis exerts beneficial effects. Although it seems feasible to modulate insulin sensitivity and subsequent cardiovascular risk factors by decreasing the total amount of dietary fat and increasing the proportion of polyunsaturated fatty acids, additional studies on the efficacy of specific fatty acids, dietary fibre, and low-energy diets, as well as on the mechanisms involved are required to understand the real function of these dietary components. Finally, dietary supplements containing folate and vitamins

B6 and/or B12 should be tested for their potential to reduce cardiovascular risk by lowering the plasma level of homocysteine.

### **Folate deficiencies and cardiovascular pathologies**

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Clinical Chemistry and Laboratory Medicine (Germany), 1998, 36/7 (419-429)

Although folates are widely distributed in foods, folate deficiencies may be more frequent than expected because their true availability may be impaired due to their lability under various food cooking and processing conditions. Folate deficiency is frequently observed in elderly people, smokers, alcoholics and oral contraceptive users. It is also associated with the mutation leading to the thermolabile variant of N5,10-methylenetetrahydrofolate reductase which is observed in about 10% of the population. In addition to the essential role of the intracellular pool of polyglutamates in de novo biosynthesis of deoxyribonucleotides which allow cell growth and division, the reduced and methylated form of folate, N5-methyltetrahydrofolate, is required for the remethylation of homocysteine to methionine. By inhibiting this remethylation pathway, folate deficiency induces homocysteine efflux into the circulation. Many studies have shown a negative correlation between plasma folate, particularly N5-methyltetrahydrofolate, and circulating homocysteine levels. In addition, folate deficiency is a major cause of hyperhomocysteinemia which is fully recognised as an independent risk factor for atherothrombosis. Epidemiological and recent experimental studies have demonstrated that folate deficiency might increase the risk of cardiovascular disease by increasing circulating homocysteine levels. Thus, the clinical efficiency of folate supplementation, especially N5-methyltetrahydrofolate, in reducing homocysteine-dependent cardiovascular risk should be evaluated.

### **Homocysteine vs cholesterol: Competing views, or a unifying explanation of arteriosclerotic cardiovascular disease?**

Cramer D.A.

Laboratory Medicine (United States), 1998, 29/7 (410-417)

The amino acid homocysteine is getting increased recognition as cholesterol's partner in causing heart attacks and strokes. The mounting clinical evidence makes it likely that screening tests for this factor will be part of routine laboratory workups. Learn what homocysteine does to arteries and how to lower your risk of developing coronary artery disease.

## **Hyperhomocysteinemia and atherosclerotic vascular disease: Pathophysiology, screening, and treatment**

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Archives of Internal Medicine (United States), 1998, 158/12 (1301-1306)

Hyperhomocysteinemia has recently been identified as an important risk factor for atherosclerotic vascular disease. This article reviews homocysteine metabolism, causes of hyperhomocysteinemia, the pathophysiological findings of this disorder, and epidemiological studies of homocysteine and vascular disease. Screening for hyperhomocysteinemia should be considered for patients at high risk for vascular disease or abnormalities of homocysteine metabolism. For primary prevention of vascular disease, treatment of patients with homocysteine levels of 14 micromol/L or higher should be considered. For secondary prevention, treatment of patients with homocysteine levels of 11 micromol/L or higher should be considered. Treatment is most conveniently administered as a folic acid supplement (400-1000 microg) and a high-potency multivitamin that contains at least 400 microg of folate. Higher doses of folic acid and cyanocobalamin supplements may be required in some patients. Until prospective clinical trial data become available, these conservative recommendations provide a safe, effective, and evidence-based approach to the diagnosis, evaluation, and management of patients with hyperhomocysteinemia.

## **Emerging approaches in the prevention of atherosclerotic cardiovascular diseases**

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International Journal of Clinical Practice, Supplement (United Kingdom), 1998, -  
/94 (7-19)

This presentation reviews data from epidemiologic and clinical trials on antioxidant vitamins, angiotensin-converting enzyme inhibitors, and homocysteine and their effect on atherosclerotic cardiovascular disease. Each of these areas seems promising, but the results of large, on-going studies must be determined before definitive conclusions can be made as to the effectiveness of these therapies.

## **Homocyst(e)inemia and risk of atherosclerosis: A clinical approach to evaluation and management**

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Endocrinologist (United States), 1998, 8/3 (170-177)

Elevated plasma concentrations of total homocysteine (homocyst(e)ine) are associated with increased risk of coronary artery disease, cerebrovascular disease, peripheral vascular disease, and thrombosis. The relationship between plasma homocyst(e)ine concentrations and risk of atherosclerosis is independent and is graded even for values between the 50th and 95th percentiles.

Hyperhomocyst(e)inemia has been detected in 10 to 40% of subjects with myocardial infarction, and it appears to be most prevalent among such individuals with normal or low plasma cholesterol levels. Although a cause and effect link between homocyst(e)ine and atherosclerosis has not been established, several lines of evidence suggest that homocysteine is atherogenic and not merely a marker for increased risk. Potential mechanisms by which homocyst(e)ine might contribute to atherogenesis include direct cytotoxic effects, generation of reactive oxygen species, diminished release of nitric oxide (a primary mediator of endothelium-dependent vasodilation), endothelial dysfunction, potentiation of LDL oxidation, stimulation of smooth muscle cell proliferation, and possible abnormalities in platelet function. Screening for hyperhomocyst(e)inemia is indicated in all individuals with atherosclerosis or a strong family history of arterial occlusive disease. Folic acid deficiency is a common cause of elevated plasma homocyst(e)ine concentrations, particularly among subjects with mutations in the gene for methylenetetrahydrofolate reductase. Deficiencies of vitamins B6 and B12 also can contribute to hyperhomocyst(e)inemia. Successful treatment of hyperhomocyst(e)inemia usually is accomplished by increasing intake of folic acid above 400 to 800 microg daily, with the addition of vitamins B6 and B12 if indicated. Although lowering of plasma homocyst(e)ine levels has not been proven to reduce risk of atherosclerosis, the treatment is relatively safe and inexpensive and is expected to provide benefit. Thus, there are not compelling reasons not to provide therapy. Within the next few years, the results of intervention trials are expected to become available that will help substantiate the anticipated effects of treatment on atherosclerotic risk.

### **High homocysteine, low folate, and low vitamin B6 concentrations**

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Transplantation (United States), 1998, 65/4 (544-550)

Background: A high plasma homocysteine concentration is a risk factor for atherosclerosis and thrombosis, which are major causes of morbidity and mortality in heart transplant patients. High homocysteine concentrations may be

caused by lower folate and vitamin B6 levels. We hypothesized that these patients might have high homocysteine concentrations and low levels of folate and vitamin B6, which could contribute to the development of vascular complications.

**Methods:** Total fasting plasma homocysteine was measured in 189 cardiac transplant recipients and in healthy controls, as were concentrations of folate, vitamin B12, vitamin B6, and creatinine.

**Results:** Homocysteine concentrations were higher in recipients than controls (19.1 plus or minus 13.0 vs. 11.0 plus or minus 3.0 micromol/L,  $P < 0.01$ ), and hyperhomocysteinemia ( $>90$ th percentile for controls, 14.6 micromol/L) was seen in 68% of recipients ( $P < 0.01$ ). Folate and vitamin B6 concentrations were lower (5.9 plus or minus 4.2 vs. 7.9 plus or minus 4.2 pmol/L and 40 plus or minus 25 vs. 84 plus or minus 77 nmol/L, respectively;  $P < 0.01$  for both). Folate and vitamin B6 deficiencies were seen in 10.8% and 17.9% of recipients, respectively ( $P < 0.01$ ). Hyperhomocysteinemia was more frequent in patients with vascular complications after transplantation than in those without (79.2% vs. 63.8%,  $P < 0.05$ ).

**Conclusions:** Elevated plasma homocysteine and deficiencies of folate and vitamin B6 are common in transplant recipients. A high homocysteine concentration was more common in patients with vascular complications. Prospective studies are now required to evaluate the role of these abnormalities as risk factors for the atherothrombotic complications of transplantation.

### **Recommended dietary allowance of folic acid sufficient for low homocysteine level**

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**Objective.** To determine the effect of short term supplementation of vitamin B6 (pyridoxine) followed by folic acid in apparently healthy volunteers on the fasting plasma homocysteine concentrations (hyperhomocysteinemia is an independent risk factor for premature atherosclerosis).

**Design.** Prospective, descriptive.

**Setting.** Academic Hospital Groningen, the Netherlands.

**Methods.** Apparently healthy Dutch volunteers, aged 20-75 years, were supplemented with vitamin B6 1 mg/kg/day during 7 days followed by folic acid 5 mg/day during another 7 days. On days 0, 7 and 14 the fasting plasma homocysteine concentrations were measured. A change of an individual's plasma

homocysteine level was considered statistically significant if the change in percentage exceeded 2.8 times the sum of the analytical and the intraindividual biological variation.

Results. There were 103 participants, 45 males and 58 females, with average ages of 43 and 44 years, respectively (on day 7, data were available on 101 participants). Baseline folic acid concentration of all participants were above the lower limit of the reference range. Eight and two of the them had vitamin B6 and vitamin B12 concentrations below the reference range, respectively. Plasma homocysteine was inversely related to plasma levels of folic acid and vitamin B12 at that moment. During vitamin B6 supplementation the mean plasma homocysteine level did not change; one participant exhibited a significant plasma homocysteine decrease. During folic acid supplementation the mean plasma homocysteine decreased from 11.7 micromol/l (SD: 5.6) to 9.1 (SD: 3.4); 40 participants (40%) exhibited significant plasma homocysteine decreases. At the end of the study plasma homocysteine was still related to plasma vitamin B12.

Conclusion. The folic acid status of the participants at baseline was not associated with the lowest plasma homocysteine levels. Since atherosclerosis risk may increase continuously with decreasing plasma homocysteine, it may be wise to keep plasma homocysteine levels as low as possible. To reach this goal, the recommended dietary allowance of folic acid may have to be increased.

### **Homocysteine and cardiovascular disease**

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Annual Review of Medicine (United States), 1998, 49/- (31-62)

An elevated level of total homocysteine (tHcy) in blood, denoted hyperhomocysteinemia, is emerging as a prevalent and strong risk factor for atherosclerotic vascular disease in the coronary, cerebral, and peripheral vessels, and for arterial and venous thromboembolism. The basis for these conclusions is data from about 80 clinical and epidemiological studies including more than 10,000 patients. Elevated tHcy confers a graded risk with no threshold, is independent of but may enhance the effect of the conventional risk factors, and seems to be a particularly strong predictor of cardiovascular mortality.

Hyperhomocysteinemia is attributed to commonly occurring genetic and acquired factors including deficiencies of folate and vitamin B12. Supplementation with B-vitamins, in particular with folic acid, is an efficient, safe, and inexpensive means to reduce an elevated tHcy level. Studies are now in progress to establish whether such therapy will reduce cardiovascular risk.

### **Vitamin supplementation reduces blood homocysteine levels: A controlled trial in patients with venous thrombosis and healthy volunteers**

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Arteriosclerosis, Thrombosis, and Vascular Biology (United States), 1998, 18/3 (356-361)

Hyperhomocysteinemia is a risk factor for atherosclerosis and thrombosis and is inversely related to plasma folate and vitamin B12 levels. We assessed the effects of vitamin supplementation on plasma homocysteine levels in 89 patients with a history of recurrent venous thrombosis and 227 healthy volunteers. Patients and hyperhomocysteinemic (homocysteine level >16 micromol/L) volunteers were randomized to placebo or high-dose multivitamin supplements containing 5 mg folic acid, 0.4 mg hydroxycobalamin, and 50 mg pyridoxine. A subgroup of volunteers without hyperhomocysteinemia was also randomized into three additional regimens of 5 mg folic acid, 0.5 mg folic acid, or 0.4 mg hydroxycobalamin. Before and after the intervention period, blood samples were taken for measurements of homocysteine, folate, cobalamin, and pyridoxal-5'-phosphate levels. Supplementation with high-dose multivitamin preparations normalized plasma homocysteine levels (less than or equal to 16 micromol/L) in 26 of 30 individuals compared with 7 of 30 in the placebo group. Also in normohomocysteinemic subjects, multivitamin supplementation strongly reduced homocysteine levels (median reduction, 30%; range, -22% to 55%). In this subgroup the effect of folic acid alone was similar to that of multivitamin: median reduction, 26%; range, -2% to 52% for 5 mg folic acid and 25%; range, -54% to 40% for 0.5 mg folic acid. Cobalamin supplementation had only a slight effect on homocysteine lowering (median reduction, 10%; range, -21% to 41%). Our study shows that combined vitamin supplementation reduces homocysteine levels effectively in patients with venous thrombosis and in healthy volunteers, either with or without hyperhomocysteinemia. Even supplementation with 0.5 mg of folic acid led to a substantial reduction of blood homocysteine levels.

### **Hyperhomocysteinemia - A new risk factor for atherosclerosis**

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Klinikerarzt (Germany), 1998, 27/3 (64-71)

Prospective and case control studies have shown that a mild elevation of plasma homocysteine represents a risk factor for coronary, cerebrovascular and peripheral arterial disease. Hyperhomocysteinemia is a result of a combination of genetic and dietary factors. The mechanisms by means of which raised plasma

homocysteine leads to vascular disease are not fully understood, and are presently undergoing intensive investigation. It appears that homocysteine has an effect on endothelial cells, smooth muscle cells in the vessel wall, the connective tissue matrix of atherosclerotic plaques, blood platelets and coagulation factors, and also plays a role in the oxidative modification of lipids and lipoproteins. Folic acid supplementation, either alone or in combination with vitamin B6 reduces or normalises increased homocysteine levels. The optimal dosage and combination of the B vitamins is, however not yet known. Ongoing long-term prospective, randomized and placebo-controlled studies are presently investigating the question whether this treatment can also exert a positive influence on the incidence and progression of atherosclerotic vascular disease, possibly even reducing the morbidity and mortality of these diseases. In clinical practice, however, mild hyperhomocysteinaemia should already be included in the differential diagnostic consideration of atherosclerotic risk factors. In view of its low level of side-effects treatment of patients with mild hyperhomocysteinaemia with folic acid and pyridoxal phosphate, would, however already appear justified, even though no information is presently available as to its longterm effects.

### **Homocysteine and vascular diseases**

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Hematologie (France), 1998, 4/1 (7-16)

Homocysteine is metabolized through 2 pathways: transsulfuration leading to the formation of cystathionine via cystathionine beta synthase (CbetaS) and its co-factor, pyridoxal 5' phosphate (vitamin B6); remethylation forming methionine via methionine synthase and its coenzyme methylcobalamin, the methyl donor being methyltetrahydrofolate (methylTHF) derived from the reduction of methylene THF via methylenetetrahydrofolate reductase (MTHFR). The increase of homocysteine is an independent risk factor for vascular diseases; indeed hyperhomocysteinemia is toxic for the endothelial cell. The increase of homocysteine is due - to genetic factors: CbetaS or MTHFR deficiency, defective synthesis of active forms of cobalamins - nutritional factors such as folate, vitamin B12 or B6 deficiencies; - some diseases mainly chronic renal insufficiency. In congenital diseases associated with severe hyperhomocysteinemia and huge homocystinuria, the vascular lesion is characterized by precocious atherosclerosis associated to arterial and venous thromboembolism. Besides, numerous epidemiological studies have shown the relationship between moderate hyperhomocysteinemia and the occurrence of vascular diseases, cerebral, coronary, peripheral artery diseases, venous thrombosis. In addition, hyperhomocysteinemia is a predictive risk factor of vascular diseases or even of mortality. There is a relation between plasma homocysteine levels and folate, vitamin B6 and B12 levels from one part, and plasma homocysteine levels and a mutation on the gene of MTHFR C677 right arrow T, which in an homozygous state, usually induces an increase of plasma

homocysteine levels. Folic acid alone or in association with vitamin B12 and B6 decreases and often normalizes homocysteine levels. Folic acid supplementation could be an effective treatment, inexpensive and not toxic for the prevention of some vascular diseases.

### **Effects of folic acid supplementation on hyperhomocysteinemia in CAPD patients: Effects on unsaturated fatty acids**

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Japanese Journal of Nephrology (Japan), 1998, 40/1 (8-16)

Hyperhomocysteinemia has been recognized as one of the risk factors for atherosclerosis and premature vascular disease. Patients on dialysis and end-stage renal disease also manifest high plasma concentrations of homocysteine. We performed this study to evaluate the effects of folic acid supplementation on hyperhomocysteinemia in CAPD patients. Twenty-three CAPD patients (8 males, 15 females, 49.1 plus or minus 14.2-years-old) dialyzed for 22.7 plus or minus 19.2 months participated in the study. Daily 5-mg doses of folic acid supplementation for 4 weeks significantly reduced plasma concentrations of total homocysteine ( $p < 0.01$ ) and serine ( $p < 0.001$ ). This observation suggests that the reduction of plasma concentrations of total homocysteine results from activation of homocysteine remethylation to methionine. On the other hand, folic acid supplementation also revealed significant correlations between changes in serum concentrations of both dihomo-gamma-linolenic acid and arachidonic acid and changes in plasma concentrations of total homocysteine ( $r = -0.517$ ,  $p < 0.05$ ,  $r = -0.451$ ,  $p < 0.05$ , respectively). In addition, serum concentrations of both dihomo-gamma-linolenic acid and arachidonic acid in 11 CAPD patients with hyperhomocysteinemia (less than or equal to 35 micromol/liter) were significantly lower than those of 12 CAPD patients with normohomocysteinemia (<35 micromol/liter) ( $p < 0.05$ , respectively). Serum concentrations of both dihomo-gamma-linolenic acid and arachidonic acid in CAPD patients with hyperhomocysteinemia increased significantly ( $p < 0.01$ ,  $p < 0.05$ , respectively) and reached similar levels of CAPD patients with normohomocysteinemia, while plasma concentrations of total homocysteine decreased after folic acid supplementation. These findings suggest that correction of hyperhomocysteinemia in patients on dialysis produces an increase in unsaturated fatty acids.

### **Low circulating folate and vitamin B6 concentrations risk factors for stroke, peripheral vascular disease, and coronary artery disease**

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Circulation (United States), 1998, 97/5 (437-443)

**Background-** A high plasma homocysteine concentration is a risk factor for atherosclerosis, and circulating concentrations of homocysteine are related to levels of folate and vitamin B6. This study was performed to explore the interrelationships between homocysteine, B vitamins, and vascular diseases and to evaluate the role of these vitamins as risk factors for atherosclerosis.

**Methods-** In a multicenter case-control study in Europe, 750 patients with documented vascular disease and 800 control subjects frequency- matched for age and sex were compared. Plasma levels of total homocysteine (before and after methionine loading) were determined, as were those of red cell folate, vitamin B12, and vitamin B6.

**Results-** In a conditional logistic regression model, homocysteine concentrations greater than the 80th percentile for control subjects either fasting (12.1 micromol/L) or after a methionine load (38.0 micromol/L) were associated with an elevated risk of vascular disease independent of all traditional risk factors. In addition, concentrations of red cell folate below the lowest 10th percentile (<513 nmol/L) and concentrations of vitamin B6 below the lowest 20th percentile (<23.3 nmol/L) for control subjects were also associated with increased risk. This risk was independent of conventional risk factors and for folate was explained in part by increased homocysteine levels. In contrast, the relationship between vitamin B6 and atherosclerosis was independent of homocysteine levels both before and after methionine loading.

**Conclusions-** Lower levels of folate and vitamin B6 confer an increased risk of atherosclerosis. Clinical trials are now required to evaluate the effect of treatment with these vitamins in the primary and secondary prevention of vascular diseases.

### **Vitamins B6, B12, and folate: Association with plasma total homocysteine and risk of coronary atherosclerosis**

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Journal of the American College of Nutrition (United States), 1998, 17/5 (435-441)

**Objectives:** To investigate the association of status of vitamins B6, B12 and folate with plasma fasting total homocysteine (tHcy) and with risk of coronary atherosclerosis; and to establish whether associations between vitamins and risk of coronary atherosclerosis are mediated by tHcy.

**Methods:** The study population consisted of 131 patients with angiographically-defined severe coronary atherosclerosis and 88 referents with no or minor

coronary stenosis. Previous analyses in this study population have shown that fasting tHcy is an independent risk factor for coronary atherosclerosis. In the present analyses, using multiple linear regression, we estimated differences in tHcy concentrations between subjects in the lowest and highest quartiles of concentrations of each of the vitamins, adjusting for age, gender, total:HDL cholesterol ratio, smoking habits, alcohol intake, blood pressure, serum creatinine, body mass index and the two other vitamins. We used logistic regression analysis conditional on the set of potential confounders described above to study the association between vitamin concentration and risk of coronary atherosclerosis. By comparing these estimated odds ratios (ORs) with those that were additionally adjusted for fasting tHcy, we determined whether the vitamins exerted their effects on disease risk via homocysteine metabolism.

Results: Cases who were in the upper quartile of serum vitamin B12 and erythrocyte folate concentrations showed statistically significantly lower tHcy concentrations (-4.00 and -4.71 micromol/L, respectively) than those in the lowest quartile. Referents in the upper quartile of plasma B6 showed significantly lower tHcy concentrations (-2.36 micromol/L) than referents in the lowest quartile. Subjects in the lowest quartile of vitamin B12 concentration had higher risk of coronary atherosclerosis (OR: 2.91; 95% CI: 1.10, 7.71) compared to those in the highest quartile. The ORs and 95% CIs for low B6 and low folate were 0.86 (95% CI: 0.33, 2.22) and 0.58 (95% CI: 0.23, 1.48), respectively. Additional adjustment for fasting tHcy weakened associations, although data indicated that low vitamin B12 concentration is a risk factor for coronary atherosclerosis, independently of tHcy.

Conclusion: The presently accepted view that vitamin B6 mainly affects tHcy after methionine loading, and not fasting tHcy, is contradicted by our findings in referents. Low vitamin B12 concentrations were associated with an increased risk of coronary atherosclerosis, partly independently of tHcy. Although low folate status was a strong determinant of elevated tHcy concentrations, it was not associated with increased risk of coronary atherosclerosis.

### **Prospective study of coronary heart disease incidence in relation to fasting total homocysteine, related genetic polymorphisms, and B vitamins: The atherosclerosis risk in communities (ARIC) study**

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Background - Elevated plasma total homocysteine (tHcy), low B-vitamin intake, and genetic polymorphisms related to tHcy metabolism may play roles in coronary heart disease (CHD). More prospective studies are needed.

Methods and Results - We used a prospective case-cohort design to determine whether tHcy-related factors are associated with incidence of CHD over an average of 3.3 years of follow-up in a biracial sample of middle-aged men and women. Age-, race-, and field center-adjusted CHD incidence was associated positively ( $P < 0.05$ ) with tHcy in women but not men, and CHD was associated negatively ( $P < 0.05$ ) with plasma folate (women only), plasma pyridoxal 5'-phosphate (both sexes), and vitamin supplementation (women only). However, after accounting for other risk factors, only plasma pyridoxal 5'-phosphate was associated with CHD incidence; the relative risk for the highest versus lowest quintile of pyridoxal 5'-phosphate was 0.28 (95% CI=0.1 to 0.7). There was no association of CHD with the C677T mutation of the methylenetetrahydrofolate reductase gene or with 3 mutations of the cystathionine beta-synthase gene.

Conclusions - Our prospective findings add uncertainty to conclusions derived mostly from cross-sectional studies that tHcy is a major, independent, causative risk factor for CHD. Our findings point more strongly to the possibility that vitamin B6 offers independent protection. Randomized trials, some of which are under way, are needed to better clarify the interrelationships of tHcy, B vitamins, and cardiovascular disease.

### **Hypothesis: Cis-unsaturated fatty acids as potential anti-peptic ulcer drugs**

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Prostaglandins Leukotrienes and Essential Fatty Acids (United Kingdom), 1998, 58/5 (377-380)

It is now reasonably well established that *Helicobacter pylori* is the most likely cause for duodenal ulcer. What is not clear is how this infection is related to the excess acid production, why few people with *Helicobacter pylori* infection have duodenal ulcer and how diet is related to duodenal ulcer. Here it is suggested that a deficiency of cis-unsaturated fatty acids (otherwise called as polyunsaturated fatty acids, PUFAs) especially gamma-linolenic acid, dihomo-gamma-linolenic acid, arachidonic acid and eicosapentaenoic acid may be responsible for duodenal ulcer. Patients with active duodenal ulcer are known to have low concentrations of these PUFAs in their plasma phospholipid fraction and they revert to normal levels after treatment with H2 blockers. In addition, these PUFAs have the ability to inhibit the growth of *Helicobacter pylori*, suppress acid production and both in experimental animals and humans these PUFAs could heal the ulcer and protect the gastric mucosa from aspirin and steroid-induced damage. Further, PUFAs have other beneficial actions such as capacity to prevent/arrest atherosclerosis, lower plasma cholesterol and triglyceride levels and cytotoxic action on tumour cells. Since PUFAs can be administered over long periods of time and are relatively non-toxic, it is suggested that PUFAs may be exploited as potential anti-ulcer agents.

### **Influence of long-chain polyunsaturated fatty acids on oxidation of low density lipoprotein**

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Prostaglandins Leukotrienes and Essential Fatty Acids (United Kingdom), 1998, 59/2 (143-151)

Enrichment of low density lipoprotein (LDL) with long-chain fatty acids, such as eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) found in fish oil, is thought to increase its oxidative susceptibility although such an increase has not been clearly demonstrated. The purpose of this study was to determine the composition and fatty acid concentration of LDL obtained from postmenopausal women given a supplement of fish oil and relate these values to its oxidative susceptibility. Fish oil supplementation significantly increased LDL concentration of EPA ( $P = 0.0001$ ) and DHA ( $P = 0.0001$ ) and decreased that of linoleic acid ( $P = 0.006$ ). The concentration of free cholesterol, cholesterol ester, phospholipids and protein was unchanged while triglyceride concentration increased 8% ( $P = 0.02$ ).  $\text{Cu}^{2+}$ -mediated oxidation resulted in a shorter lag time, slower oxidation rate and similar concentrations of conjugated dienes of EPA/DHA-enriched LDL than EPA/DHA-unenriched LDL. Stepwise multiple regression indicated that the primary predictor of oxidative susceptibility of LDL was linoleic acid, even after enrichment with EPA and DHA. The oxidation rate of EPA/DHA-unenriched LDL correlated with the cholesteryl ester concentration ( $P = 0.003$ ) while that of EPA/DHA-enriched correlated with the concentration of phospholipids ( $P = 0.03$ ). These data suggest that EPA/DHA-enriched LDL have decreased oxidative susceptibility and that surface lipids may mediate its rate of oxidation.

### **Effects of eicosapentaenoic acids on remnant-like particles, cholesterol concentrations and plasma fatty acid composition in patients with diabetes mellitus**

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In Vivo (Greece), 1998, 12/3 (311-314)

Remnant lipoproteins are transient metabolites from chylomicron and/or very low density lipoproteins (VLDL), and remnant hyperlipoproteinemia has recently been reported to be a risk factor for atherosclerosis. Eicosapentaenoic acid (EPA), a major component of fish oil, has the following effects: anti-platelet aggregation, vaso-dilation, anti-inflammation, hypotriglyceridemia, and therefore has potential anti-atherosclerotic effects. We measured serum of remnant-like particle

cholesterol (RLP-C) concentrations, and investigated the effects of EPA on serum RLP-C concentrations in patients with diabetes mellitus. Ten patients with non-insulin dependent diabetes mellitus were treated with 900-1800 mg EPA ethyl-ester daily for 3 months. We investigated serum RLP-C concentrations and plasma fatty acid composition before and after the administration of EPA. Serum RLP-C concentrations were significantly decreased 3 months after the administration of EPA (from 14.5 plus or minus 5.3 mg/dL to 3.3 plus or minus 0.8 mg/dL,  $P < 0.01$ ). Plasma EPA concentrations and the ratios of EPA to arachidonic acids (AA) were significantly increased during the same period (from 86.2 plus or minus 12.4 mg/L to 194.6 plus or minus 27.3 mg/L,  $P < 0.01$ , from 0.571 plus or minus 0.074 to 1.242 plus or minus 0.163,  $P < 0.01$ , respectively). Serum RLP-C concentrations were inversely correlated with the ratios of EPA to AA in plasma ( $r = -.516$ ,  $P < 0.05$ ). These results suggested that administration of EPA was effective on remnant hyperlipoprote nemia which was a risk factor for atherosclerosis.

### **Omega-3 ethyl ester concentrate decreases total apolipoprotein CIII and increases antithrombin III in postmyocardial infarction patients**

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Clinical Drug Investigation (New Zealand), 1998, 15/6 (473-482)

This study investigated whether an ethyl ester preparation of fish oil (omega-3) could normalise raised plasma concentrations of triglycerides, apolipoprotein CIII on apolipoprotein B-containing particles (LP CIII:B) found in patients with recent acute myocardial infarction. We also studied the effect of fish oil on antithrombin III levels. Out of 75 patients with a plasma triglyceride value less than or equal to 2.0 mmol/L, 22 normalised their triglycerides during diet and were therefore not randomised. The remaining patients were randomly assigned to 12 weeks' treatment with a daily dose of 4g omega-3 or placebo. Mean plasma triglyceride concentrations were reduced by 24% from 3.10 plus or minus 1.15 (SD) to 2.53 plus or minus 0.94 mmol/L ( $p < 0.001$ ) on omega-3 ( $p < 0.001$  vs placebo). The reduction was due to decreases in very low density lipoprotein concentrations. Total apolipoprotein CIII decreased significantly. This was due to reductions in LP CIII:non B concentrations, but the ratio LP CIII:non B/LP CIII:B was unaffected because of a slight insignificant decrease in LP CIII:B. The plasma triglyceride decreasing effect of omega-3 could therefore not be due to redistribution of CIII between lipoproteins. Low density lipoprotein (LDL) cholesterol increased significantly with omega-3 by 7%, and antithrombin III increased significantly with fish oil. In conclusion, omega-3 had a moderate plasma triglyceride lowering effect and increased LDL cholesterol slightly, while antithrombin III increased in patients with hypertriglyceridaemia who had recently experienced a myocardial infarction. Myocardial infarction starts via a thrombotic process at an atherosclerotic lesion in a coronary artery. Most patients developing this disease have an abnormal plasma lipoprotein pattern consisting of

slightly raised triglycerides (TGs), moderately elevated total cholesterol, and low high density lipoprotein (HDL) cholesterol values predisposing to atherosclerosis. Hypertriglyceridaemia may be associated with a greater risk for thrombosis in postmyocardial infarction patients because of a reduced fibrinolytic capacity. The dyslipidaemia may also indicate an unfavourable distribution of plasma lipoprotein particles in patients with myocardial infarction. Dietary changes normalise the dyslipidaemia in some patients but are inadequate in others. In these latter patients pharmacological lipid-lowering treatment is necessary. The myocardial infarction patient with an athero-thrombogenic syndrome could theoretically therefore benefit from a pharmacological agent acting on both the thrombotic and lipidaemic pathophysiological pathways. The pharmacological potency of the omega-3-fatty acids allows for this possibility. It has been known since the mid 1970s that omega-3-fatty acids are effective in lowering plasma triglyceride concentrations. They also increase the concentration of HDL cholesterol slightly. Their effects on cholesterol have varied, with some studies showing increases and others decreases. These fatty acids also inhibit platelet aggregation. It was therefore of interest to expand the experience of this type of treatment to effects on plasma lipoprotein particle distribution. We also studied parameters of fibrinolysis since the literature shows diverging results of omega-3-fatty acids on these parameters. In the present study we tested a new compound, omega-3, an oil consisting of ethyl esters of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with the aim of normalising dyslipidaemia, and reducing the thrombotic tendency in a potentially important target population for such treatment, postmyocardial infarction patients. The high EPA and DHA concentration in omega-3 made a convenient intake of only four capsules daily possible. The design of the study followed the current guidelines for secondary prevention of ischaemic heart disease.

### **A central role for protein kinase c overactivity in diabetic glomerulosclerosis: Implications for prevention with antioxidants, fish oil, and ACE inhibitors**

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Medical Hypotheses (United Kingdom), 1998, 50/2 (155-165)

The primary etiologic factor in diabetic glomerulosclerosis appears to be an overproduction of transforming growth factor-beta by mesangial cells, which in turn reflects a hyperglycemic mediated overactivation of protein kinase C (PKC) throughout the glomerulus. Membrane-active antioxidants, fish oil, and angiotensin-converting enzyme inhibitors can act to down-regulate glomerular PKC activity, via a variety of mechanisms that may include activation of diacylglycerol kinase and suppression of phosphatidate phosphohydrolase, support of endothelial nitric oxide and heparan sulfate production, inhibition of thromboxane and angiotensin synthesis/activity, and correction of glomerular hypertension. The beneficial impact of these measures on vascular endothelial function may be of more general utility in the prevention of diabetic

complications such as retinopathy, neuropathy, and atherosclerosis. Adjunctive use of gamma-linolenic acid is indicated for prevention of neuropathy, and it is conceivable that bioactive chromium will have protective activity not solely attributable to improved glycemic control. Re-establishing euglycemia must clearly remain the core strategy for preventing diabetic complications, but when glycemic control remains suboptimal, practical, safe measures are at hand for decreasing risk.

### **Taurine in management of diffuse cerebral arteriopathy. Clinical and electroencephalographic observations, and mental test results**

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Clin.Ter. (Italy), 1974, 71/5 (427-436)

Following previous work on the subject, a number of patients suffering from cerebral arteriopathy were treated with Taurine by mouth (3-4 g a day, for some weeks). The patients were subdivided into 3 groups, according to the main presenting symptom. The treatment was in every case well tolerated. Noticeable improvement, of statistical significance, was obtained in the power of concentration, of memory and of orientation. The best results were obtained in those patients who presented intermittent confusional episodes, while least success was obtained in those with severe intellectual deterioration and in the most elderly.

### **Effect of taurine on incipient senile involution of the brain**

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Clin.Ter. (Italy), 1974, 70/5 (425-433)

The use of taurine was investigated in the treatment of early mental deterioration from senile involution (so called cerebral arteriosclerosis). It was carried out as a double blind trial using a placebo. Psychometric tests were used to evaluate its effects objectively. Statistical evaluation showed that taurine can produce amelioration of the early symptoms of senile mental deterioration, suggesting that it can be used to treat, or as a prophylactic for, mental involution in the pre senile and senile age groups.

### **Ginkgo - Myth and reality**

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Schweiz. Rundsch. Med. Prax. (Switzerland), 1995, 84/1 (1-6)

Ginkgo biloba is one of the oldest, still existing plants. Extracts from its leaves were already used in ancient China whereas in the Western World, they have been utilized only since the Sixties when it became technically possible and feasible to isolate the essential substances of Ginkgo biloba. Pharmacologically, there are two groups of substances which are of some significance: the flavonoids, effective as oxygen-free radical scavengers, and the terpenes (i.e. the ginkgolides) with their highly specific action as platelet activating factor (PAF) inhibitors. Clinically important indications for Ginkgo biloba extracts are cerebral insufficiency and atherosclerotic disease of peripheral arteries of intermediate severity. In several placebo-controlled clinical studies, symptoms of cerebral insufficiency have been effectively and significantly influenced. Most of these investigations have examined the efficacy of Ginkgo biloba extracts such as EGb 761 and LI 1370.

### **Phytotherapy in cardiovascular diseases. Supportive therapy in early stages**

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Therapiewoche (Germany), 1994, 44/29 (1650-1653)

Circulatory effective herbs such as haw-thorn, ginkgo and garlic have a very important share in the entire phytotherapy. Due to their good tolerance they are used in less severe classes of the frequent circulatory system's diseases as arteriosclerosis and consequent symptoms, heart failure, orthostatic dysfunction.

### **Evaluation of the evidence on the role of tomato products in disease prevention**

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Proceedings of the Society for Experimental Biology and Medicine (United States), 1998, 218/2 (140-143)

During the last 30 years, research in the field of nutrition and chronic disease causation has led to exciting, significant progress in providing an understanding of specific risk factors and chemopreventive agents. The major health problems considered are cardiovascular diseases and the nutritionally linked cancers, including those in the stomach, colon, breast, prostate, ovary, and endometrium. The major elements considered were salt, type and amount of fat, and heterocyclic amines formed during cooking. Bran cereal fiber, as well as vegetables, fruits, and

tea have been shown to inhibit the complex processes of initiation and development of these diseases. One aspect involved in initiation and development of both cardiovascular diseases and the cancers noted are abnormal oxidative processes leading to the generation of hydroxy radicals and peroxy compounds. In part, the protective role of vegetables, fruits, and tea is to provide antioxidant vitamins and specific polyphenols that display a powerful inhibition in oxidative reactions. Epidemiological studies as well as laboratory experimentation have yielded sound data and evidence in support of the fact that vegetables, fruits, and tea and specific antioxidants therein account mechanistically for inhibition. Geographic pathology has provided important data that populations with a regular intake of tomato products, such as in the Mediterranean region, have a lower incidence of the chronic diseases noted. The current Symposium is considering the varied mechanisms of action of tomato products in general, and one of the active principles, lycopene. Cooking is a factor in releasing the desirable antioxidants from tomatoes. Cooked tomato products may be preferable to the raw vegetable or juices derived from tomatoes bearing on absorption of the active principles. Optimally, absorption of lycopene, a highly lipid-soluble chemical, is improved in the presence of a small, but essential amount of oil or fat. Research in the field of nutrition and health has shown that monounsaturated oils such as olive oil or canola oil are most desirable, since such oils do not increase the risk of atherosclerosis, coronary heart disease, or the nutritionally linked cancers. The International Symposium on tea conducted in 1991 has provided worldwide interest in research on the beneficial effects of tea. It is now hoped that the present Symposium, dealing with another inexpensive and readily available food, tomatoes, will enhance interest in and funding for additional research, to underwrite future recommendations for possibly enhanced production and use of tomato-derived nutritional elements, with the goal of application to the prevention of major chronic diseases, the treatment of which is costly and often ineffective.

### **Copper: An antioxidant nutrient for cardiovascular health**

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Curr. Opin. Lipidology (United Kingdom), 1994, 5/1 (22-28)

Dietary copper often is low in the Western diet; low intakes may affect all stages of atherosclerosis adversely. Impaired oxidative defense in copper deficiency contributes to hypercholesterolemia, hypertension, and impaired prostaglandin metabolism. Free copper ion does not exist in vivo; some in-vitro experiments are conducted with millions-fold excesses.

### **Trace elements and cardiovascular diseases**

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*Acta Pharmacol. Toxicol.* (Denmark), 1986, 59/Suppl. 7 (317-324)

Evidence linking marginal intakes of the trace elements, chromium, copper, zinc and selenium, with abnormal lipid metabolism and ultimately cardiovascular diseases is accumulating from both animal and human studies. Chromium supplementation of normal adult men, as well as diabetics, has been reported to increase high density lipoprotein cholesterol and decrease triglycerides and total cholesterol. Subjects with the highest total cholesterol and triglycerides usually respond the most to supplemental chromium. Improvements in lipid metabolism, as well as those in glucose metabolism, appear to be related to improvements in insulin efficiency due likely to increased receptor number. Animal studies also indicate that improvements in serum cholesterol, aortic lipids and plaque formations due to supplemental chromium are associated with decreased circulating insulin. Insufficient dietary copper also leads to elevated lipid levels and impaired heart function. Animal studies indicate an obvious degradation of the heart muscles. Zinc appears to function in cardiovascular diseases primarily via its antagonism with copper. Selenium may also affect cardiovascular diseases since selenium is postulated to be involved in platelet aggregation. These data demonstrate that the trace elements, chromium, copper, and selenium, have beneficial effects on risk factors associated with cardiovascular diseases suggesting that a decreased risk of cardiovascular disease may be achieved by adequate intake of trace elements.

### **Increased cholesterol in plasma in a young man during experimental copper depletion**

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*Metab. Clin. Exp.* (USA), 1984, 33/12 (1112-1118)

Signs of copper depletion were produced in a healthy man by an amount of dietary copper (0.83 mg/day) similar to that in some contemporary diets. Urinary and fecal loss of copper exceeded intake. Plasma copper, ceruloplasmin and superoxide dismutase activity in erythrocytes decreased. Cholesterol in plasma increased, and hematologic indices were unchanged. Lipid metabolism may be a more sensitive index of copper nutriture than are changes in hematology. The findings support the hypothesis that inadequate copper nutriture or altered copper metabolism contributes to the occurrence of ischemic heart disease.

### **Antioxidant vitamin levels in plasma and low density lipoprotein of obese girls**

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Free Radical Research (United Kingdom), 1998, 28/1 (81-86)

To investigate the antioxidant status of obese children, we analyzed beta-carotene and alpha-tocopherol levels in plasma and low density lipoprotein (LDL). We also analyzed the fatty acid composition of LDL as a substrate for oxidative stress. The plasma beta-carotene and alpha-tocopherol levels were relatively lower in obese girls than in normal controls. However, the plasma alpha-tocopherol/lipids ratio was significantly lower in obese girls than in normal controls. Both LDL beta-carotene and LDL alpha-tocopherol levels were significantly lower in obese girls than in normal controls, although no obvious differences were observed in plasma levels. In obese girls LDL contained more polyunsaturated fatty acid (PUFA) compared with normal controls. When the peroxidizability Index (PI) was calculated to estimate the susceptibility of lipids to oxidative stress, obese girls had significantly higher PI values than normal controls. Both the LDL beta-carotene/PI ratio and the LDL alpha-tocopherol/PI ratio were significantly lower in obese girls than in normal controls. These results indicate the increased susceptibility of LDL to oxidative stress in obese girls which may promote atherosclerosis later in life.

### **Dietary iron concentration alters LDL oxidatively the effect of antioxidants**

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Research Communications in Molecular Pathology and Pharmacology (United States), 1998, 99/1 (69-80)

Low-density lipoprotein (LDL) cholesterol participates in the atherosclerotic process only after oxidative modification (o-LDL). Persons with elevated body iron concentrations are at higher risk of atherosclerosis. Iron in vitro is capable of oxidizing LDL, but it is unknown whether or not high dietary iron concentrations alter LDL in vivo. The aim of this study was, therefore, to investigate (i) whether dietary iron concentrations cause LDL-cholesterol oxidation and (ii) whether antioxidants can prevent such changes. Rats received diets differing only in iron concentration: 35 mg/kg, 150 mg/kg or 300 mg/kg diet. A LDL-VLDL particle was isolated and the following parameters measured: malondialdehyde and lipid hydroperoxide concentrations (as an indication for lipid peroxidation); alpha-tocopherol and retinol concentrations (as antioxidants); protein sulfhydryl and carbonyl concentrations (as an indication of protein modification); agarose gel electrophoresis and cholesterol/protein ratio. Dietary iron increased LDL- VLDL lipid peroxidation (malondialdehyde and lipid hydroperoxide concentrations), protein modification (sulfhydryl concentration), agarose migration distance and band width as well as cholesterol/protein ratio. Increased quantities of dietary iron led to a higher degree of oxidative change in LDL-VLDL. Lipid peroxidation, as

well as protein modification, occurred, suggesting apoB changes. This was probably due to diminished antioxidant concentrations of alpha-tocopherol and beta-carotene. Antioxidant supplementation (alpha-tocopherol and beta-carotene), however, prevented all the above changes and could be helpful in the prevention of atherosclerosis.

### **Plasma antioxidant and trace element status in familial hypercholesterolemic patients treated with LDL-apheresis**

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Annales Pharmaceutiques Francaises (France), 1998, 56/1 (18-25)

Oxidation of low density lipoprotein is involved in the pathogenesis of atherosclerosis. Epidemiological studies suggest a negative correlation between the occurrence of cardiovascular diseases and blood concentrations of lipophilic antioxidants such as vitamin A and E and beta-carotene. Trace elements such as selenium, zinc and copper are involved in the activity of antioxidant enzymes: glutathione peroxidase and superoxide dismutase. The aim of this work was to determine the antioxidant and trace elements status of patients with very severe hypercholesterolemia and who were treated by dextran sulphate low density lipoprotein apheresis, in comparison with two control populations: one constituted by normocholesterolemic subjects and the other by hypercholesterolemic patients before treatment. Our results showed that, as compared with normocholesterolemic subjects, patients treated by LDL-apheresis were not deficient in vitamin E, beta-carotene and copper but had low plasma levels of selenium, zinc and vitamin A. The low selenium and vitamin A levels were due to the treatment by LDL-apheresis by itself, while the hypercholesterolemia of these patients might have provoked the low plasma levels of zinc. This study pointed out the interest of a supplement of selenium, zinc and vitamin A in patients treated by LDL-apheresis.

### **Ascorbic acid clearance in diabetic nephropathy**

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Journal of Diabetes and its Complications (United States), 1998, 12/5 (259-263)

The incidence of cardiovascular disease is increased in diabetic nephropathy. Increased oxidative stress in diabetes is believed to play an important role in the pathogenesis of atherosclerosis in diabetes. Since antioxidant vitamins, such as ascorbic acid, often are reduced in diabetes, we hypothesized that the renal

clearance of ascorbic acid is increased in patients with diabetic nephropathy. Thirty-seven subjects with diabetic nephropathy were studied: 18 had microalbuminuria (30-300 mg/day albuminuria); the remainder had clinical nephropathy (> 300 mg/day albuminuria). Indices of glycemic control (glucose, hemoglobin A(1C)) and renal function (albuminuria and creatinine clearance) were measured in addition to serum and urinary ascorbic acid levels. Results showed that subjects with clinical nephropathy had lower mean plasma ascorbic acid ( $p = 0.0009$ ) and higher renal clearance of ascorbic acid ( $p = 0.005$ ) than those with microalbuminuria. Bivariate analysis revealed an inverse correlation between creatinine clearance and AA clearance ( $r = -0.42$ ,  $p = 0.009$ ). There was a significant linear association between the quantity of albuminuria and ascorbic acid clearance ( $r = 0.49$ ,  $p = 0.002$ ). Thus, patients with diabetic nephropathy have reduced ascorbic acid levels due to increased ascorbic acid clearance. The decrease in antioxidant defense that arises from the low levels of vitamin C may contribute to the increased cardiovascular morbidity and mortality observed in this population.

### **Vascular damage from smoking: Disease mechanisms at the arterial wall**

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Vascular Medicine (United Kingdom), 1998, 3/1 (21-28)

The products of tobacco combustion are absorbed into the systemic circulation. Absorbed nicotine stimulates the release of catecholamines, whilst other products (perhaps including nicotine) injure the arterial endothelium and promote atherogenesis. Free radicals and aromatic compounds diminish the endothelial synthesis of nitric oxide, causing impaired endothelium-dependent relaxation of arteries, the earliest clinical sign of endothelial dysfunction. Smoking alters the shear forces and rheology at the endothelial surface and these changes enhance the effects of products of tobacco combustion to upregulate leucocyte adhesion molecules on the endothelial surface. The increased oxidation of low density lipoprotein (LDL) in smokers has synergistic effects to promote monocyte adhesion and monocyte migration into the subintimal space. Continued stimulation of intimal cells by oxidized LDL leads to the development of atherosclerosis. Many of these effects are ameliorated by high concentrations of vitamin C. Smoking also potentiates thrombosis at the dysfunctional endothelium by increasing the concentration of plasma fibrinogen and altering the activity of platelets. All these pro-atherogenic effects of smoking to injure the endothelium also are observed, albeit to lesser extent, in passive smokers.

### **Dynamics of vitamin E action against LDL oxidation**

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Free Radical Research (United Kingdom), 1998, 28/6 (561-572)

Vitamin E acts as an important antioxidant against oxidative modification of low density lipoprotein (LDL) which is accepted as an initial event in the pathogenesis of atherosclerosis. In spite of the numerous studies and reports, the action and role of vitamin E have not been fully elucidated yet. In this brief overview, the dynamics of action of vitamin E as an antioxidant have been discussed and it is emphasized that the total antioxidant potency is determined by the relative importance of many competing reactions which is determined by the reactivities and concentrations of substrates, radicals and antioxidant and by physical factors of the environment.

### **Cost-effectiveness of vitamin E therapy in the treatment of patients with angiographically proven coronary narrowing (CHAOS trial)**

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American Journal of Cardiology (United States), 1998, 82/4 (414-417)

Epidemiologic studies have suggested that vitamin E (alpha-tocopherol) may play a preventive role in reducing the incidence of atherosclerosis. The aim of this paper was to conduct a cost-effectiveness analysis of vitamin E supplementation in patients with coronary artery disease using data from the Cambridge Heart Antioxidant Study (CHAOS). The study compared cost-effectiveness in the context of Australian and United States (US) health care utilization. The main clinical outcome used in the economic evaluation was the incidence of acute myocardial infarction (AMI) which was nonfatal. Utilization of health care resources was estimated by conducting a survey of Australian clinicians and published Australian and US cost data. Cost savings of \$127 (A\$181) and \$578/patient randomized to vitamin E therapy compared with patients receiving placebo were found for Australian and US settings, respectively. Savings in the vitamin E group were due primarily to reduction in hospital admissions for AMI. This occurred because the vitamin E group had a 4.4% lower absolute risk of AMI than did the placebo group. Less than 10% of health care costs in the Australian evaluation was due to vitamin E (\$150 [A\$214/patient]). Our economic evaluation indicates that vitamin E therapy in patients with angiographically proven atherosclerosis is cost-effective in the Australian and US settings.

### **Alpha-Tocopherol induces oxidative damage to DNA in the presence of copper(II) ions**

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Chemical Research in Toxicology (United States), 1998, 11/8 (855-862)

There is currently much interest in the possibility that dietary antioxidants may confer protection from certain diseases, such as atherosclerosis and cancer. The importance of alpha-tocopherol (vitamin E) as a biological antioxidant is widely recognized. However, pro-oxidant properties of alpha-tocopherol have been observed in chemical systems, and it has been reported that the vitamin can induce tumor formation and act as a complete tumor promotor in laboratory animals. In the present communication, we find that alpha-tocopherol can act as a potent DNA-damaging agent in the presence of copper(II) ions, using a simplified, in vitro model. alpha-Tocopherol was found to promote copper-dependent reactive oxygen species formation from molecular oxygen, resulting in DNA base oxidation and backbone cleavage. Neither alpha-tocopherol nor Cu(II) alone induced DNA damage. Bathocuproine, a Cu(I)-specific chelator, and catalase inhibited the DNA damage, whereas free hydroxyl radical scavengers did not. The order of DNA cleavage sites was thymine, cytosine > guanine residues. Examinations using an oxygen electrode and cytochrome c indicate that molecular oxygen was consumed in the reaction of alpha-tocopherol and Cu(II) and that superoxide was formed. Stoichiometry studies showed that two Cu(II) ions could be reduced by each alpha-tocopherol molecule. Electron spin resonance spin-trapping investigations were then used to demonstrate that hydrogen peroxide interacts with Cu(I) to generate the reactive species responsible for DNA damage, which is either the hydroxyl radical or a species of similar reactivity. These findings may be of relevance to the tumorigenic properties of the vitamin reported in the literature. However, further studies are required to establish the significance of these reactions under in vivo conditions.

### **Erythrocyte antioxidant status in asymptomatic hypercholesterolemic men**

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Atherosclerosis (Ireland), 1998, 138/2 (375-381)

An imbalance between antioxidant and oxidant-generating systems leading to an oxidative stress has already been proposed in the pathogenesis of atherosclerosis. In the present study we investigated the antioxidant status in 60 asymptomatic hypercholesterolemic (HC) men compared with 48 normocholesterolemic (NC) men. Hypercholesterolemic subjects had a significantly lower red blood cell vitamin E (vit E-RBC) content in spite of their normal total plasma and HDL vitamin E concentrations. Activities of erythrocyte superoxide dismutase and glutathione peroxidase were not significantly different between groups. We also determined the resistance of RBCs to an oxidative stress by determining the extent of hemolysis induced by a water-soluble azo-compound. This resistance

was significantly decreased in HC men compared with NC subjects. These results demonstrate an altered antioxidant status of RBC in asymptomatic HC men associated with an increased erythrocyte susceptibility to an oxidative stress. The measure of the vitamin E content in RBC might be the most sensitive parameter for evidencing early oxidative stress which does not need an adaptation of enzymatic protective systems.

### **Monocyte superoxide production is inversely related to normal content of alpha-tocopherol in low-density lipoprotein**

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Atherosclerosis (Ireland), 1998, 138/2 (263-269)

Vitamin E (alpha-tocopherol) is a potent peroxy radical scavenger. According to the oxidative theory of atherosclerosis, it prevents oxidation of low-density lipoprotein (LDL) and thereby lowers the risk of cardiovascular disease. It also mediates cell actions, and specifically decreases monocyte superoxide anion-production ( $O_2^-$ -production), which is involved in LDL oxidation. We investigated whether alpha-tocopherol-containing LDL decreases this production in a manner dependent on the LDL alpha-tocopherol content (the alpha-tocopherol/apB molar ratio) in human, phorbol ester-stimulated, adherent monocytes. We found that  $O_2^-$ -production was inhibited by native LDL (n-LDL) in a manner highly sensitive to the increasing alpha-tocopherol content (range 4.5-8). In addition: (1) inhibition was greater when alpha-tocopherol was associated to acetylated LDL (ac-LDL), the maximal percentage of inhibition being 80% as opposed to 35% for n-LDL; (2) the alpha-tocopherol overloading of either form of LDL did not produce further inhibition; (3) the free form of alpha-tocopherol produced lower inhibition compared with the lipoprotein-associated forms; (4) inhibition was not related to the cell content of alpha-tocopherol. We propose that the cell targeting of alpha-tocopherol is crucial to the inhibition of monocyte  $O_2^-$ -production, and thus that the role of normal LDL-alpha-tocopherol contents (range 6-8) in the prevention of atherogenic processes needs to be reexamined.

### **Dehydroepiandrosterone protects low density lipoproteins against peroxidation by free radicals produced by gamma-radiolysis of ethanol-water mixtures**

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Atherosclerosis (Ireland), 1998, 136/1 (99-107)

Oxidized low density lipoproteins (LDL) are believed to play a central role in the events that initiate atherosclerosis. Antioxidants have been shown to decrease the oxidation of LDL, leading to the diminution of atherosclerosis. Since it is well-known that decreased levels of dehydroepiandrosterone (DHEA) are linked to the development of atherosclerosis, we studied the modulation of the oxidation of LDL by DHEA. LDL were obtained from 10 healthy subjects and oxidized by free radicals produced by gamma-radiolysis of ethanol-water mixtures. The formation of conjugated dienes and thiobarbituric acid-reactive substances (TBARS), the vitamin E content, as well as the incorporation of 4-[14C]DHEA in LDL and the chemotactic effect of oxidized LDL in the presence of DHEA towards monocytes, were investigated. It was found that DHEA was able to inhibit the oxidation of LDL by reducing over 90% of the conjugated dienes and TBARS formation, as well as by reducing the vitamin E disappearance and significantly decreasing the chemotactic activity towards monocytes. Our results suggest that DHEA exerts its antioxidative effect by protecting the endogenous vitamin E of LDL.

### **Oxidation of low density lipoproteins in the pathogenesis of atherosclerosis**

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Atherosclerosis (Ireland), 1998, 137/Suppl. (S33-S38)

Malondialdehyde (MDA)-modified and oxidized low density lipoproteins (LDL) have been demonstrated in atherosclerotic lesions. Elevated titers of autoimmune antibodies specific for MDA-modified LDL predicted the progression of carotid atherosclerosis and of myocardial infarction. Recently, elevated levels of MDA-modified LDL were detected in the plasma of patients with ischemic heart disease, whereas, elevated levels of oxidized LDL were detected in the plasma of patients with ischemic heart disease and of heart transplant patients with post-transplant cardiovascular disease. Although increased levels of autoimmune antibodies against oxidatively modified LDL and increased levels of oxidized LDL antigen appear to be associated with atherosclerotic cardiovascular disease, there is to date no direct proof of the causal role of oxidized LDL in atherothrombosis. However, the decreased risk of cardiovascular disease associated with the administration of antioxidants (e.g. vitamin E), estrogen supplementation and increased levels of high density lipoproteins (HDL) may, at least partially, be due to the inhibition of oxidation of LDL or to the reversal of the atherothrombotic effects of oxidized LDL.

### **Vitamin E in diabetes mellitus**

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Medizinische Welt (Germany), 1998, 49/5 (250-255)

There is growing evidence that supplementation with vitamin E in higher doses has a protective role in prevention of atherosclerosis. Patients with diabetes mellitus have an increased risk of cardiovascular complications and oxidative stress plays a promoting role in developing of long-term diabetic late complications. Low vitamin E status is a risk factor for diabetes mellitus. Therefore, an increased need for antioxidative substances, especially vitamin E, is given. The results prove that a therapy with adjuvant vitamin E in higher doses e.g. Pexan E (R) may lead to a regression of diabetic late complications. Epidemiological and prospective clinical studies indicate that high vitamin E levels may be associated with decreased cardiovascular diseases. For patients with diabetes mellitus the supplementation with vitamin E in higher doses is to be recommended in particular.

#### **Metabolic consequences of reduced plasma LDL-C during hypolipidaemic therapy: Assessment of lipoperoxidation activity and vitamin E in lipoprotein fractions**

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Klinicka Biochemie a Metabolismus (Czech Republic), 1998, 6/2 (77-81)

There is evidence that statins may have other anti-atherogenic effects besides their lipid-lowering activity, including effects on oxidability of lipoproteins. Thus the aim of the present study was to examine consequences of reduced plasma cholesterol during hypolipidaemic therapy, lipoperoxidation activity and the distribution of the antioxidant vitamin E in lipoprotein fractions. A group of 14 patients (8 men, 6 women, age 35-65y) with hypercholesterolaemia was treated using simvastatin (Zocor (R) MSD, 20 mg daily). Blood samples were examined before treatment, after 4 and 8 weeks of therapy. After ultracentrifugation, samples were analyzed for vitamin E content in lipoprotein fractions. Antioxidant status was examined using serum thiobarbituric acid reacting substance (TBARS) activity. Simvastatin reduced both total cholesterol (9.28plus or minus0.56 vs. 6.64plus or minus0.35 mmol/l;  $p < 0.001$ ), IDL-C (1.76plus or minus0.15 vs. 1.08plus or minus0.09 mmol/l;  $p < 0.001$ ), and LDL-C (3.80plus or minus0.35 vs. 2.63plus or minus0.23 mmol/l;  $p < 0.001$ ). Total serum vitamin E was reduced during hypolipidaemic therapy (44.54plus or minus3.62 vs. 36.85plus or minus1.72 micromol/l;  $p = 0.06$ ). However, the ratio of serum vitamin E/total serum cholesterol (4.86plus or minus0.31 vs. 5.63plus or minus0.28 micromol/mmol;  $p = 0.09$ ) and the ratio of LDL-C vitamin E/LDL-C (3.57plus or minus0.31 vs. 3.67plus or minus0.31 micromol/mmol; n.s.) did not change, and the ratio of IDL-C vitamin E/IDL-C (4.44plus or minus0.32 vs. 5.40plus or minus0.61 micromol/mmol;  $p < 0.01$ ) and HDL- C vitamin E/HDL-C (3.76plus or

minus0.41 vs. 5.83plus or minus0.49 micromol/mmol; p = 0.01) increased significantly. Serum TBARS decreased significantly (6.97plus or minus0.69 vs. 4.72plus or minus0.48 micromol/l; p<0.001). We conclude that effective hypolipidaemic treatment with simvastatin is associated with improved antioxidant status and a proportional increase in the serum content of vitamin E in the HDL- and IDL-cholesterol fraction, and that the content of vitamin E in total and LDL-cholesterol did not change despite the decreased concentration of its lipid carrier. With regard to functions of vitamin E, this may be an additional anti-atherogenic effect of such therapy.

### **Where are we with vitamin E?**

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Journal of Thrombosis and Thrombolysis (Netherlands), 1998, 5/3 (209-214)

Oxidative modification of low-density lipoproteins appears to significantly enhance their role in atherogenesis. Inhibition of this process with naturally occurring antioxidants has been proposed as a mechanism to retard the progression of coronary artery disease. Vitamin E has been among those natural antioxidants found to reduce atherosclerotic lesion formation in animal models. Further supported by a substantial accumulation of observational epidemiologic data demonstrating an association between antioxidant vitamin intake and reduced risk of cardiovascular mortality vitamin E has been examined in a number of case-control and prospective cohort studies as a potential agent in the primary and secondary prevention of morbidity and mortality from coronary artery disease. These efforts have generated a large body of evidence suggesting a protective role, but conflict in the data remains. In addition, even with large, well-conducted prospective epidemiologic studies, the potential effects of residual confounding may be on the same order of magnitude as the reported benefit. The several small randomized interventional trials and two larger placebo-controlled studies that have been completed to date leave some key questions unanswered. Currently ongoing are several large randomized interventional trials that will serve to further clarify the role of this promising agent in the primary and secondary prevention of atherosclerotic coronary disease.

### **The antioxidative effects of the isoflavan glabridin on endogenous constituents of LDL during its oxidation**

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Atherosclerosis (Ireland), 1998, 137/1 (49-61)

The effect of the consumption of glabridin, an isoflavan isolated from *Glycyrrhiza glabra* (licorice) root, on the susceptibility of low density lipoprotein (LDL) to oxidation was studied in atherosclerotic apolipoprotein E deficient (Edegreemice) and was compared with that of the known flavonoids, quercetin and catechin. Glabridin inhibitory activity on in vitro oxidation of human LDL was also investigated by determining the formation of lipid peroxides and oxysterols and the consumption of LDL-associated lipophilic antioxidants. Determination of the extent of LDL oxidation by measuring the formation of thiobabaturic acid reactive substances (TBARS) after 2 h of LDL incubation with CuSO<sub>4</sub> (10 microM) or 2,2'-azobis (2-amidino-propane) dihydrochloride (AAPH) (5 mM), revealed that glabridin or quercetin consumption resulted in a 53 and 54% reduction in copper ion induced oxidation, respectively, and a 95 and 83% reduction in AAPH induced LDL oxidation, respectively. No inhibition was obtained with consumption of catechin. About 80% of glabridin was found to bind to the LDL human particle. In the in vitro oxidation of LDL induced by AAPH (5 mM), glabridin inhibited the formation of TBARS, lipid peroxides and cholesteryl linoleate hydroperoxide (CLOOH) at all the concentrations tested (5-60 microM), while in oxidation induced by copper ions (10 microM), glabridin exhibited a pro-oxidant activity at concentrations lower than 20 microM, and a clear antioxidant activity at concentrations greater than 20 microM. Glabridin (30 microM) inhibited the formation of cholest-5-ene-3,7-diol (7-hydroxycholesterol), cholest-5-ene-3-ol-7-one (7-ketocholesterol) and cholestan-5,6-epoxy-3-ol (5,6-epoxycholesterol) after 6 h of AAPH induced LDL oxidation, by 55, 80 and 40%, respectively, and after 6 h of copper ion induced LDL oxidation, by 73, 94 and 52%, respectively. Glabridin also inhibited the consumption of beta-carotene and lycopene by 38 and 52%, respectively, after 0.5 h of LDL oxidation with AAPH, but failed to protect vitamin E. The in vivo and in vitro reduction of the susceptibility of LDL to oxidation obtained with glabridin, may be related to the absorption or binding of glabridin to the LDL particle and subsequent protection of LDL from oxidation by inhibiting the formation of lipid peroxides and oxysterols, and by protecting LDL associated carotenoids.

### **Effect of vitamin E and beta carotene on the incidence of primary nonfatal myocardial infarction and fatal coronary heart disease**

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Archives of Internal Medicine (United States), 1998, 158/6 (668-675)

Backgrounds: Oxidized low-density lipoprotein is involved in the pathogenesis of atherosclerosis. In epidemiological studies antioxidants have been inversely related with coronary heart disease. Findings from controlled trials are inconclusive.

**Methods:** We studied the primary preventive effect of vitamin E (alpha tocopherol) and beta carotene supplementation on major coronary events in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, a controlled trial undertaken primarily to examine the effect of these agents 50 to 69 years with no history of myocardial infarction were randomly assigned to receive vitamin E (50 mg), beta carotene (20 mg), both agents, or placebo daily for 5 to 8 years (median, 6.1 years). The end point was the first major coronary event, either nonfatal myocardial infarction (surviving at least 28 days; n=1204) or fatal coronary heart disease (n=907).

**Results:** The incidence of primary major coronary events decreased 4% (95% confidence interval, -12% to 4%) among recipients of vitamin E and increased 1% (95% confidence interval, -7% to 10%) among recipients of beta carotene compared with the respective nonrecipients. Neither agent affected the incidence of nonfatal myocardial infarction. Supplementation with vitamin E decreased the incidence of fatal coronary heart disease by 8% (95% confidence interval, - 19% to 5%), but beta carotene had no effect on this end point.

**Conclusions:** Supplementation with small dose of vitamin E has only marginal effect on the incidence of fatal coronary heart disease in male smokers with no history of myocardial infarction, but no influence on nonfatal myocardial infarction. Supplementation with beta carotene has no primary preventive effect on major coronary events.

### **Low-density lipoprotein oxidation and vitamins E and C in sustained and white-coat hypertension**

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Hypertension (United States), 1998, 31/2 (621-626)

Low-density lipoprotein oxidation and antioxidant vitamins E and C were investigated in white-coat hypertension in comparison with sustained hypertension and normotension. We selected 21 sustained hypertensive subjects, 21 white-coat hypertensive subjects, and 21 normotensive subjects matched for gender, age, and body mass index. White-coat hypertension was defined as clinical hypertension and daytime ambulatory blood pressure <139/90 (subjects were also reclassified using 134/90 and 135/85 mm Hg as cutoff points for daytime blood pressure). Blood samples were drawn for lipid profile determination, assessment of fluorescent products of lipid peroxidation in native LDL, evaluation of susceptibility to LDL oxidation in vitro (lag phase and propagation rate), and determination of LDL vitamin E and plasma vitamins E and C contents. Compared with sustained hypertensive subjects, white-coat hypertensives had significantly lower fluorescent products of lipid peroxidation (15.4plus or minus3.4 versus 10.2 plus or minus3 units of relative

fluorescence/mg LDL protein,  $P < .05$ ), longer lag phase (54 plus or minus 10 versus 88 plus or minus 10 minutes,  $P < .05$ ), lower propagation rate (8.2 plus or minus 2.5 versus 5.95 plus or minus 2.1 nmol diene/min per mg LDL cholesterol,  $P < .05$ ), higher LDL vitamin E content (8.3 plus or minus 1.1 versus 10.1 plus or minus 1.8 nmol/mg LDL cholesterol,  $P < .05$ ), and plasma vitamin C content (40 plus or minus 13 versus 57 plus or minus 9 micromol/L,  $P < .05$ ). No significant difference was observed between white-coat hypertensive and normotensive subjects. The results did not change after reclassification of subjects. Our data show that white-coat hypertensive subjects do not show an enhanced propensity to LDL oxidation or reduction in antioxidant vitamins. Given the role of LDL oxidation in the development of atherosclerosis and that of vitamin E and C in protecting against it, these findings suggest that white-coat hypertension per se carries a low atherogenic risk.

### **Progress in cardiology**

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Medecine et Hygiene (Switzerland), 1998, 56/2191 (16-20)

Coronary angioplasty is now one of the most important techniques in cardiology. Stents represent a partial solution to the frequent problem of restenosis. Hopes for prevention rest on new antiplatelet and antioxidant agents, intracoronary radiotherapy and gene therapy. Efficacy of low-molecular-weight heparin is equivalent to conventional heparin for the treatment of DVT, pulmonary embolism and unstable angina. Moreover it has the advantage of subcutaneous administration and absence of coagulation control. Unstable coronary syndrome are very common. In addition to conventional antiischemic drugs and heparin, IIb/IIa antagonists represent a promising contribution. The role of antioxidants for coronary prevention remains controversial but vitamin E appears to be useful.

### **Effect of supplementation with vitamin E on LDL oxidizability and prevention of atherosclerosis**

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BioFactors (Netherlands), 1998, 7/1-2 (51-54)

Supplementation of LDL with vitamin E is thought to protect LDL from oxidative modification and prevent the development of atherosclerosis. Large epidemiological studies have revealed that vitamin E levels in plasma are inversely correlated to the incidence of coronary heart disease. Double-blind placebo-controlled trials have reported that supplementation with vitamin E

decreases the incidence of coronary events in coronary heart disease (CHD) patients. However, it is not clear how high a dose of vitamin E is needed to prevent formation of atherosclerosis. In animal studies, a diet containing 0.125% vitamin E increased its levels in plasma two-fold and prevented formation of early atherosclerotic lesions in the thoracic aorta of hypercholesterolemic rabbits. Dose-response studies in humans have reported that 400 IU/day vitamin E increased its levels in plasma two-fold and prolonged the lag time before LDL oxidation. It has been reported that oxidizability of LDL was correlated to the atherosclerotic score of coronary angiography in CHD patients. About 400 IU/day vitamin E, which increases its levels two-fold and prolongs sufficiently the lag time before LDL oxidation, might be beneficial in decreasing the individual risk of CHD.

### **Action of vitamin E as antioxidant against oxidative modification of low density lipoprotein**

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BioFactors (Netherlands), 1998, 7/1-2 (41-50)

The hypothesis that the oxidative modification of LDL is a key event for development of atherosclerosis was originally put forward to explain the recruitment of monocytes and the accumulation of lipid-laden macrophage-derived foam cells in the fatty streak lesion (1). Subsequent studies demonstrated a large number of biological properties of oxidized LDL that could make it more atherogenic than native LDL (2). Several kinds of oxidation products in the oxidation of LDL have been identified and purified, and their biological properties investigated (3-9). These oxidation products contribute to the development of atherosclerotic lesions not only directly by inducing expression of binding molecules (10,11), cell growth (8,9) and monocyte chemotaxis (12-14) but also indirectly by inducing the release of several kinds of cytokines (13,15) for which cytotoxicity of oxidized LDL is responsible. Findings which were accumulated through these studies are summarized in Scheme 1. It has been also shown that antioxidants could attenuate the effects of these oxidative modifications of LDL. Although the relative importance of these oxidative modifications *in vivo* remains to be established, it is well-established in several experimental animal models that the administration of antioxidant can retard the development of early atherosclerotic lesions (16-20). Polyclonal and monoclonal antibodies against oxidized LDL have been established and gave positive reactions in Watanabe heritable hyperlipidemia (WHHL) rabbit and human atherosclerotic lesions (21-27).

### **Atherogenic lipoproteins support assembly of the prothrombinase complex and thrombin generation: Modulation by oxidation and vitamin E**

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Blood (United States), 1998, 91/2 (508-515)

The importance of lipoproteins in the etiology of atherosclerosis is well established. Evidence is now accumulating to implicate thrombin in the pathogenesis of atherosclerosis. We have investigated whether atherogenic lipoproteins can support thrombin generation. In the absence of platelets or endothelial cells, both very low-density lipoprotein (VLDL) and oxidized low-density lipoprotein (LDL) support assembly of the prothrombinase complex and generation of thrombin. Thrombin generation (per microg of apolipoprotein) supported by VLDL was 19.4-fold greater than that supported by high-density lipoprotein (HDL),  $P < .00001$ , and 11.7-fold greater than that supported by LDL,  $P < .00001$ . Oxidation of LDL increased lipoprotein-supported thrombin generation 12-fold compared to unmodified LDL,  $P < .0001$ . We have shown that the phenomenon of lipoprotein-supported thrombin generation is mediated predominantly by specific phospholipids and is enhanced by oxidation of these phospholipids. The addition of vitamin E (alpha-tocopherol) markedly reduced the increase in thrombin generation observed after oxidation of LDL (822 plus or minus 57 v 138 plus or minus 47 nmol/L;  $P < .0001$ ). These effects suggest that lipoproteins are important in the production of thrombin and that vitamin E may confer protection from the detrimental effects of lipoprotein oxidation by limiting thrombin formation. These results suggest that atherogenic lipoproteins are linked to the development of atherosclerosis in part by their capacity to support thrombin generation.

**A double-blind crossover study in moderately hypercholesterolemic men that compared the effect of aged garlic extract and placebo administration on blood lipids.**

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A double-blind crossover study comparing the effect of aged garlic extract with a placebo on blood lipids was performed in a group of 41 moderately hypercholesterolemic men [cholesterol concentrations 5.7-7.5 mmol/L (220-290 mg/dL)]. After a 4-wk baseline period, during which the subjects were advised to adhere to a National Cholesterol Education Program Step I diet, they were started on 7.2 g aged garlic extract per day or an equivalent amount of placebo as a dietary supplement for a period of 6 mo, then switched to the other supplement for an additional 4 mo. Blood lipids, blood counts, thyroid and liver function measures, body weight, and blood pressure were followed over the entire study period. The major findings were a maximal reduction in total serum cholesterol of 6.1% or 7.0% in comparison with the average concentration during the placebo

administration or baseline evaluation period, respectively. Low-density-lipoprotein cholesterol was also decreased by aged garlic extract, 4% when compared with average baseline values and 4.6% in comparison with placebo period concentrations. In addition, there was a 5.5% decrease in systolic blood pressure and a modest reduction of diastolic blood pressure in response to aged garlic extract. We conclude that dietary supplementation with aged garlic extract has beneficial effects on the lipid profile and blood pressure of moderately hypercholesterolemic subjects.

### **Plasma homocysteine levels and mortality in patients with coronary artery disease.**

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N Engl J Med 1997 Jul 24;337(4):230-6

Comment in: N Engl J Med 1997 Nov 27;337(22):1631-2; discussion 1632-3

**BACKGROUND:** Elevated plasma homocysteine levels are a risk factor for coronary heart disease, but the prognostic value of homocysteine levels in patients with established coronary artery disease has not been defined.

**METHODS:** We prospectively investigated the relation between plasma total homocysteine levels and mortality among 587 patients with angiographically confirmed coronary artery disease. At the time of angiography in 1991 or 1992, risk factors for coronary disease, including homocysteine levels, were evaluated. The majority of the patients subsequently underwent coronary-artery bypass grafting (318 patients) or percutaneous transluminal coronary angioplasty (120 patients); the remaining 149 were treated medically.

**RESULTS:** After a median follow-up of 4.6 years, 64 patients (10.9 percent) had died. We found a strong, graded relation between plasma homocysteine levels and overall mortality. After four years, 3.8 percent of patients with homocysteine levels below 9 micromol per liter had died, as compared with 24.7 percent of those with homocysteine levels of 15 micromol per liter or higher. Homocysteine levels were only weakly related to the extent of coronary artery disease but were strongly related to the history with respect to myocardial infarction, the left ventricular ejection fraction, and the serum creatinine level. The relation of homocysteine levels to mortality remained strong after adjustment for these and other potential confounders. In an analysis in which the patients with homocysteine levels below 9 micromol per liter were used as the reference group, the mortality ratios were 1.9 for patients with homocysteine levels of 9.0 to 14.9 micromol per liter, 2.8 for those with levels of 15.0 to 19.9 micromol per liter, and 4.5 for those with levels of 20.0 micromol per liter or higher (P for trend=0.02). When death due to cardiovascular disease (which occurred in 50 patients) was used as the end point in the analysis, the relation between homocysteine levels and mortality was slightly strengthened.

**CONCLUSIONS:** Plasma total homocysteine levels are a strong predictor of mortality in patients with angiographically confirmed coronary artery disease.

**The effect of folic acid fortification on plasma folate and total homocysteine concentrations.**

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N Engl J Med 1999 May 13;340(19):1449-54

**BACKGROUND:** In 1996, the Food and Drug Administration issued a regulation requiring all enriched grain products to be fortified with folic acid to reduce the risk of neural-tube defects in newborns. Fortification (140 microg per 100 g) began in 1996, and the process was essentially complete by mid-1997.

**METHODS:** To assess the effect of folic acid fortification on folate status, we measured plasma folate and total homocysteine concentrations (a sensitive marker of folate status) using blood samples from the fifth examination (January 1991 to December 1994) of the Framingham Offspring Study cohort for baseline values and the sixth examination (January 1995 to August 1998) for follow-up values. We divided the cohort into two groups on the basis of the date of their follow-up examination: the study group consisted of 350 subjects who were seen after fortification (September 1997 to March 1998), and the control group consisted of 756 subjects who were seen before fortification (January 1995 to September 1996).

**RESULTS:** Among the subjects in the study group who did not use vitamin supplements, the mean folate concentrations increased from 4.6 to 10.0 ng per milliliter (11 to 23 nmol per liter) ( $P < 0.001$ ) from the baseline visit to the follow-up visit, and the prevalence of low folate concentrations ( $< 3$  ng per milliliter [ $7$  nmol per liter]) decreased from 22.0 to 1.7 percent ( $P < 0.001$ ). The mean total homocysteine concentration decreased from 10.1 to 9.4 micromol per liter during this period ( $P < 0.001$ ), and the prevalence of high homocysteine concentrations ( $> 13$  micromol per liter) decreased from 18.7 to 9.8 percent ( $P < 0.001$ ). In the control group, there were no statistically significant changes in concentrations of folate or homocysteine.

**CONCLUSIONS:** The fortification of enriched grain products with folic acid was associated with a substantial improvement in folate status in a population of middle-aged and older adults.

**Dietary supplement with vitamin C prevents nitrate tolerance.**

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J Clin Invest 1998 Jul 1;102(1):67-71

Enhanced formation of superoxide radicals has been proposed to play a major role in the development of nitrate tolerance in humans. We tested the effects of vitamin C (Vit-C) supplementation on glyceroltrinitrate (GTN)-induced hemodynamic effects during 3-d nonintermittent transdermal administration of GTN (0.4 mg/h) in nine healthy subjects. Tolerance development was monitored by changes in arterial pressure, diastolic digital pulse pressure, and heart rate. Studies with GTN, Vit-C, or GTN/Vit-C were successively carried out at random in three different series in the same subjects. GTN treatment caused an immediate rise in arterial conductivity (a/b ratio of diastolic pulse), but within 2 d of initiating GTN, the a/b ratio progressively decreased and reached basal levels. In addition, there was a progressive loss of the orthostatic decrease in blood pressure. However, coadministration of Vit-C and GTN fully maintained the GTN-induced changes in the orthostatic blood pressure, and the rise of a/b ratio was augmented by 310% for the duration of the test period. Changes in vascular tolerance in GTN-treated subjects were paralleled by upregulation of the activity of isolated platelets, which was also reversed by Vit-C administration. These findings demonstrate that dietary supplementation with Vit-C eliminates vascular tolerance and concomitant upregulation of ex vivo-washed platelet activity during long-term nonintermittent administration of GTN in humans.

**Randomized, double-blind, placebo-controlled study of the preventive effect of supplemental oral vitamin C on attenuation of development of nitrate tolerance.**

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J Am Coll Cardiol 1998 May;31(6):1323-9

**OBJECTIVES:** This study sought to evaluate the preventive effect of vitamin C, an antioxidant, on the development of nitrate tolerance.

**BACKGROUND:** Decreased intracellular production of cyclic guanosine monophosphate (cGMP) is a mechanism of nitrate tolerance, and increased superoxide levels and reduced activation of guanylate cyclase have been observed in vitro.

**METHODS:** In this double-blind, placebo-controlled study, 24 normal volunteers and 24 patients with ischemic heart disease (IHD) were randomized to receive either vitamin C (2 g three times daily [vitamin C group, n=12]) or placebo (placebo group, n=12). The vasodilator response to nitroglycerin was assessed

with forearm plethysmography by measuring the change in FBF before and 5 min after sublingual administration of 0.3 mg of nitroglycerin. Blood samples were simultaneously obtained to measure platelet cGMP levels. FBF was measured, and blood sampling was performed serially at baseline (day 0), 3 days after administration of vitamin C or placebo (day 3) and 3 days after application of a 10-mg/24-h nitroglycerin tape concomitantly with oral vitamin C or placebo (day 6).

**RESULTS:** There were no differences between the vitamin C and placebo groups in percent increases in FBF (%FBF) or platelet cGMP levels (%cGMP) after administration of sublingual nitroglycerin on day 0 (%FBF: normal volunteers 31+/-8 vs. 32+/-10; patients with IHD 32+/-9 vs. 32+/-8; %cGMP: normal volunteers 37+/-9 vs. 39+/-10; patients with IHD 38+/-10 vs. 39+/-10 [vitamin C group vs. placebo group]) or day 3 (%FBF: normal volunteers 32+/-9 vs. 33+/-9; patients with IHD 31+/-10 vs. 31+/-10; %cGMP: normal volunteers 36+/-8 vs. 37+/-9; patients with IHD 39+/-11 vs. 38+/-10 [vitamin C group vs. placebo group]). The %FBF and %cGMP in the placebo group were significantly lower on day 6 than in the vitamin C group (%FBF: normal volunteers 30+/-8 vs. 19.4,  $p < 0.01$ ; patients with IHD 29+/-9 vs. 17+/-6,  $p < 0.01$ ; %cGMP: normal volunteers 36.10 vs. 17+/-6,  $p < 0.01$ ; patients with IHD 37+/-11 vs. 15+/-5,  $p < 0.01$  [vitamin C group vs. placebo group]).

**CONCLUSIONS:** These results indicate that combination therapy with vitamin C is potentially useful for preventing the development of nitrate tolerance.

### **Co-enzyme Q10: a new drug for cardiovascular disease.**

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J Clin Pharmacol 1990 Jul;30(7):596-608

Co-enzyme Q10 (ubiquinone) is a naturally occurring substance which has properties potentially beneficial for preventing cellular damage during myocardial ischemia and reperfusion. It plays a role in oxidative phosphorylation and has membrane stabilizing activity. The substance has been used in oral form to treat various cardiovascular disorders including angina pectoris, hypertension, and congestive heart failure. Its clinical importance is now being established in clinical trials worldwide.

### **Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure. CoQ10 Drug Surveillance Investigators.**

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Mol Aspects Med 1994;15 Suppl:s287-94

Digitalis, diuretics and vasodilators are considered the standard therapy for patients with congestive heart failure, for which treatment is tailored according to the severity of the syndrome and the patient profile. Apart from the clinical seriousness, heart failure is always characterized by an energy depletion status, as indicated by low intramyocardial ATP and coenzyme Q10 levels. We investigated safety and clinical efficacy of Coenzyme Q10 (CoQ10) adjunctive treatment in congestive heart failure which had been diagnosed at least 6 months previously and treated with standard therapy. A total of 2664 patients in NYHA classes II and III were enrolled in this open noncomparative 3-month postmarketing study in 173 Italian centers. The daily dosage of CoQ10 was 50-150 mg orally, with the majority of patients (78%) receiving 100 mg/day. Clinical and laboratory parameters were evaluated at the entry into the study and on day 90; the assessment of clinical signs and symptoms was made using from two-to seven-point scales. The results show a low incidence of side effects: 38 adverse effects were reported in 36 patients (1.5%) of which 22 events were considered as correlated to the test treatment. After three months of test treatment the proportions of patients with improvement in clinical signs and symptoms were as follows: cyanosis 78.1%, oedema 78.6%, pulmonary rales 77.8%, enlargement of liver area 49.3%, jugular reflux 71.81%, dyspnoea 52.7%, palpitations 75.4%, sweating 79.8%, subjective arrhythmia 63.4%, insomnia 66.2.8%, vertigo 73.1% and nocturia 53.6%. Moreover we observed a contemporary improvement of at least three symptoms in 54% of patients; this could be interpreted as an index of improved quality of life.

**Italian multicenter study on the safety and efficacy of coenzyme Q10 as adjunctive therapy in heart failure (interim analysis). The CoQ10 Drug Surveillance Investigators.**

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Clin Investig 1993;71(8 Suppl):S145-9

Digitalis, diuretics, and vasodilators are considered standard therapy for patients with congestive heart failure, for which treatment is tailored according to the severity of the syndrome and the patient profile. Apart from the clinical seriousness, heart failure is always characterized by an energy depletion status, as indicated by low intramyocardial ATP and coenzyme Q10 levels. We investigated safety and clinical efficacy of coenzyme Q10 (CoQ10) adjunctive treatment in congestive heart failure, which had been diagnosed at least 6 months previously and treated with standard therapy. A total of 2500 patients in NYHA classes II and III were enrolled in this open noncomparative 3-month postmarketing drug surveillance study in 173 Italian centers. The daily dose of CoQ10 was 50-150 mg orally, with the majority of patients (78%) receiving 100 mg/day. Clinical and

laboratory parameters were evaluated at the entry into the study and on day 90; the assessment of clinical signs and symptoms was made using from two- to seven-point scales. Preliminary results on 1113 patients (mean age 69.5 years) show a low incidence of side effects: 10 adverse reactions were reported in 8 (0.8%) patients, of which only 5 reactions were considered as correlated to the test treatment. After 3 months of test treatment the proportions of patients with improvement in clinical signs and symptoms were as follows: cyanosis 81%, edema 76.9%, pulmonary rales 78.4%, enlargement of the liver area 49.3%, jugular reflux 81.5%, dyspnea 54.2%, palpitations 75.7%, sweating 82.4%, arrhythmia 62%, insomnia 60.2%, vertigo 73%, and nocturia 50.7%.

### **Clinical experience of coenzyme Q10 to enhance intraoperative myocardial protection in coronary artery revascularization.**

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Cardiovasc Drugs Ther 1991 Mar;5 Suppl 2:297-300

Seventy-eight patients undergoing coronary artery bypass grafting (CABG) were compared retrospectively to evaluate whether pretreatment with coenzyme Q10 (CoQ) is effective in preventing left ventricular depression in early reperfusion following CABG. CoQ (5 mg/kg, intravenously) was given to 60 patients, 2 hours prior to the onset of cardiopulmonary bypass (CPB). CABG was performed using saphenous vein under CPB associated with cold cardioplegia in the standard fashion. Heart rate, mean arterial pressure, and cardiac index showed no significant difference between the CoQ and control groups. However, left ventricular stroke work index was significantly elevated at 6 and 10 hours of reperfusion following CABG in the CoQ-treated group compared with the controls. Serum MB-CK was lower at 0 and 6 hours of reperfusion in the CoQ group compared with the controls. These results suggest that pretreatment with intravenous CoQ is effective in preventing left ventricular depression in early reperfusion and in minimizing myocardial cellular injury during CABG followed by reperfusion.

### **Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis.**

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N Engl J Med 1995 Feb 2;332(5):286-91  
Comment in: N Engl J Med 1995 Feb 2;332(5):328-9  
Comment in: N Engl J Med 1995 Aug 3;333(5):325

**BACKGROUND.** Epidemiologic studies have identified hyperhomocysteinemia as a possible risk factor for atherosclerosis. We determined the risk of carotid-artery atherosclerosis in relation to both plasma homocysteine concentrations and nutritional determinants of hyperhomocysteinemia.

**METHODS.** We performed a cross-sectional study of 1041 elderly subjects (418 men and 623 women; age range, 67 to 96 years) from the Framingham Heart Study. We examined the relation between the maximal degree of stenosis of the extracranial carotid arteries (as assessed by ultrasonography) and plasma homocysteine concentrations, as well as plasma concentrations and intakes of vitamins involved in homocysteine metabolism, including folate, vitamin B12, and vitamin B6. The subjects were classified into two categories according to the findings in the more diseased of the two carotid vessels: stenosis of 0 to 24 percent and stenosis of 25 to 100 percent.

**RESULTS.** The prevalence of carotid stenosis of  $\geq 25$  percent was 43 percent in the men and 34 percent in the women. The odds ratio for stenosis of  $\geq 25$  percent was 2.0 (95 percent confidence interval, 1.4 to 2.9) for subjects with the highest plasma homocysteine concentrations ( $\geq 14.4$   $\mu\text{mol per liter}$ ) as compared with those with the lowest concentrations ( $\leq 9.1$   $\mu\text{mol per liter}$ ), after adjustment for sex, age, plasma high-density lipoprotein cholesterol concentration, systolic blood pressure, and smoking status ( $P < 0.001$  for trend). Plasma concentrations of folate and pyridoxal-5'-phosphate (the coenzyme form of vitamin B6) and the level of folate intake were inversely associated with carotid-artery stenosis after adjustment for age, sex, and other risk factors.

**CONCLUSIONS.** High plasma homocysteine concentrations and low concentrations of folate and vitamin B6, through their role in homocysteine metabolism, are associated with an increased risk of extracranial carotid-artery stenosis in the elderly.

### **Vitamin B-12, vitamin B-6, and folate nutritional status in men with hyperhomocysteinemia.**

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Am J Clin Nutr 1993 Jan;57(1):47-53

We measured the vitamin B-6, vitamin B-12, and folic acid nutritional status in a group of apparently healthy men ( $n = 44$ ) with moderate hyperhomocysteinemia (plasma homocysteine concentration  $> 16.3$   $\mu\text{mol/L}$ ). Compared with control subjects ( $n = 274$ ) with normal plasma homocysteine ( $\leq 16.3$   $\mu\text{mol/L}$ ) concentrations, significantly lower plasma concentrations of pyridoxal-5'-phosphate ( $P < 0.001$ ), cobalamin ( $P < 0.001$ ), and folic acid ( $P = 0.004$ ) were demonstrated in hyperhomocysteinemic men. The prevalence of suboptimal vitamin B-6, B-12, and folate status in men with hyperhomocysteinemia was

25.0%, 56.8%, and 59.1%, respectively. In a placebo-controlled follow-up study, a daily vitamin supplement (10 mg pyridoxal, 1.0 mg folic acid, 0.4 mg cyanocobalamin) normalized elevated plasma homocysteine concentrations within 6 wk. Because hyperhomocysteinemia is implicated as a risk factor for premature occlusive vascular disease, appropriate vitamin therapy may be both efficient and cost-effective to control elevated plasma homocysteine concentrations.

### **Carotid and femoral artery wall thickness and stiffness in patients at risk for cardiovascular disease, with special emphasis on hyperhomocysteinemia.**

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Arterioscler Thromb Vasc Biol 1998 Dec;18(12):1958-63

Recent developments in ultrasound technology enable the noninvasive measurement of structural and functional vessel wall changes. Until now, the effect of homocysteine on the arterial wall has remained unclear: reports on intima-media thickness (IMT) yield conflicting results, whereas data on vessel wall stiffness are lacking. Because several cardiovascular risk factors result in an increased IMT or stiffness, different groups at risk for atherosclerotic disease, with special emphasis on hyperhomocysteinemia, were studied. Nineteen patients homozygous and 14 subjects heterozygous for cystathionine beta-synthase (CBS) deficiency, 21 patients with familial hypercholesterolemia (FH), 15 patients with essential hypertension, 20 smokers, and 28 control subjects were studied. The IMT values (both right and left) of the common carotid artery (CCA), bulb (BUL), internal carotid artery (ICA), and common femoral artery (CFA) were measured in millimeters by high-resolution ultrasound (Biosound). The distensibility (DC, in  $10^{-3}$ . kPa $^{-1}$ ) and compliance (CC in mm $^2$ . kPa $^{-1}$ ) coefficients of the CCA (right and left) and CFA (right) were determined by a wall track system (Pie Medical). The mean IMT of the posterior wall in the CCA was 0.70 $\pm$ 0.09 mm in healthy controls. For patients with vascular disease, FH, and hypertension and in smokers, the mean CCA IMT was larger, whereas no major differences in IMT were observed in patients either homozygous or heterozygous for CBS deficiency. The DC and CC in the right CCA were 23.5 $\pm$ 6.9 ( $10^{-3}$ . kPa $^{-1}$ ) and 0.9 $\pm$ 0.3 (mm $^2$ . kPa $^{-1}$ ) in healthy subjects, slightly lower in patients homozygous for CBS deficiency, and clearly lower in patients with vascular disease, FH, and hypertension. No positive correlation was found between plasma homocysteine level and either IMT, CC, or DC. Because smoking was a confounder in each risk group, a stepwise regression analysis was carried out to assess the contribution of each risk factor on IMT and arterial wall stiffness. Age explained most of the variation in IMT of the CCA (coefficient of determination  $R^2$  of 0.34), whereas  $R^2$  values for serum low density lipoprotein cholesterol, smoking (pack-years), and systolic blood pressure were 0.08, 0.07, and 0.06, respectively. Homocysteine did not contribute to variation in IMT in

both the CCA and CFA. Age and smoking contributed to the variation in IMT in the CFA. The variation in DC and CC in the right CCA and right CFA could in part be explained by age, low density lipoprotein cholesterol, and blood pressure. Plasma homocysteine concentration explained only a small proportion of the variation in DC in the CCA ( $R^2=0.02$ ) and in CC in the CFA ( $R^2=0.04$ ). In this study, no relationship was found between homocysteine level and the thickness of the arterial wall, with only a marginal influence on stiffness.

### **Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men: a 10-year follow-up.**

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Arterioscler Thromb Vasc Biol 1998 Dec;18(12):1895-901

Hyperhomocysteinemia is an independent risk factor for atherosclerotic disease in the middle-aged. We investigated whether a high serum homocysteine level is a risk factor for vascular disease in 878 elderly men (mean age at baseline, 71.5 years; range, 64 to 84 years) in a population-based, representative cohort followed up for 10 years in Zutphen, the Netherlands. Thirty-one percent had nonfasting homocysteine levels  $\geq 17$  micromol/L. After adjustment for other major risk factors, high homocysteine levels at baseline (the third compared with the first tertile) were associated with an increased baseline prevalence of myocardial infarction (odds ratio [OR], 1.81; 95% confidence interval [CI], 1.07 to 3.08; P for trend, 0.03) and with a marginally significant increase in the risk of dying of coronary heart disease (relative risk [RR], 1.58; 95% CI, 0.93 to 2.69; P for trend, 0.09) but not with an increased risk of first-ever myocardial infarction. In addition, high homocysteine levels at baseline were associated with an increased baseline prevalence of stroke (OR, 4.61; 95% CI, 1.79 to 11.89; P for trend, 0.002) and with an increased risk of dying of cerebrovascular disease in subjects without hypertension (RR, 6.18; 95% CI, 2.28 to 16.76) but not in those with hypertension. High homocysteine levels were associated with an increased risk of first-ever stroke among normotensive subjects that was not statistically significant (RR, 1.77 [95% CI, 0.83 to 3.75; P for trend, 0.14]). In a general population of elderly men, a high homocysteine level is common and is strongly associated with the prevalence of coronary heart disease and cerebrovascular disease. It is a strong predictive factor for fatal cerebrovascular disease in men without hypertension but less so for coronary heart disease.

### **Vitamin supplementation reduces blood homocysteine levels: a controlled trial in patients with venous thrombosis and healthy volunteers.**

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*Arterioscler Thromb Vasc Biol* 1998 Mar;18(3):356-61

Hyperhomocysteinemia is a risk factor for atherosclerosis and thrombosis and is inversely related to plasma folate and vitamin B12 levels. We assessed the effects of vitamin supplementation on plasma homocysteine levels in 89 patients with a history of recurrent venous thrombosis and 227 healthy volunteers. Patients and hyperhomocysteinemic (homocysteine level >16 micromol/L) volunteers were randomized to placebo or high-dose multivitamin supplements containing 5 mg folic acid, 0.4 mg hydroxycobalamin, and 50 mg pyridoxine. A subgroup of volunteers without hyperhomocysteinemia was also randomized into three additional regimens of 5 mg folic acid, 0.5 mg folic acid, or 0.4 mg hydroxycobalamin. Before and after the intervention period, blood samples were taken for measurements of homocysteine, folate, cobalamin, and pyridoxal-5'-phosphate levels. Supplementation with high-dose multivitamin preparations normalized plasma homocysteine levels (< or = 16 micromol/L) in 26 of 30 individuals compared with 7 of 30 in the placebo group. Also in normohomocysteinemic subjects, multivitamin supplementation strongly reduced homocysteine levels (median reduction, 30%; range, -22% to 55%). In this subgroup the effect of folic acid alone was similar to that of multivitamin: median reduction, 26%; range, -2% to 52% for 5 mg folic acid and 25%; range, -54% to 40% for 0.5 mg folic acid. Cobalamin supplementation had only a slight effect on homocysteine lowering (median reduction, 10%; range, -21% to 41%). Our study shows that combined vitamin supplementation reduces homocysteine levels effectively in patients with venous thrombosis and in healthy volunteers, either with or without hyperhomocysteinemia. Even supplementation with 0.5 mg of folic acid led to a substantial reduction of blood homocysteine levels.

### **Plasma homocysteine levels related to interactions between folate status and methylenetetrahydrofolate reductase: a study in 52 healthy subjects.**

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*Metabolism* 1998 Nov;47(11):1413-8

Hyperhomocysteinemia, a risk factor for vascular disease, is related to vitamin B12, vitamin B6, and especially folate deficiency, or to genetic factors such as mutations in methylenetetrahydrofolate reductase (MTHFR), an enzyme involved in the remethylation pathway of homocysteine to methionine. Recently, a C677 --> T mutation identified in the MTHFR gene was found to be frequently associated with decreased MTHFR activity and an elevated plasma homocysteine concentration. Since hyperhomocysteinemia seems to be determined by both genetic and environmental factors, we studied the interactions between MTHFR (phenotype and genotype) and folate status, including methyltetrahydrofolate

(methylTHF), the product of MTHFR, on the homocysteine concentration in 52 healthy subjects, (28 women and 24 men; mean age, 32.7 years). MTHFR activity seems to be dependent on folate status, as shown by a lower activity in folate-deficient subjects and a return to normal values after supplementation with folic acid, and also by a decreased enzymatic activity on phytohemagglutinin (PHA)-stimulated lymphocytes grown in a folic acid-deficient medium. Conversely, the C677 --> T mutation seems to influence folate metabolism. Subjects who were homozygous for this mutation (+/+) had significantly higher plasma homocysteine and lower plasma folate and total and methylfolate levels in red blood cells (RBCs) than heterozygous (+/-) and normal (-/-) subjects. The ratio of RBC methylfolate to RBC total folate was, respectively, 0.27 in +/+, 0.66 in +/-, and 0.71 in -/-. This mutation seems to have an impact on methylTHF generation. These data illustrate the interactions between nutritional and genetic factors.

### **Effectiveness of low-dose crystalline nicotinic acid in men with low high-density lipoprotein cholesterol levels.**

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Arch Intern Med 1996 May 27;156(10):1081-8

**BACKGROUND:** Hypoalphalipoproteinemia (low serum concentration of high-density lipoprotein cholesterol [HDL-C]) is a common pattern of dyslipidemia associated with coronary heart disease. High doses of nicotinic acid effectively raise HDL-C levels in this condition, but they are commonly accompanied by side effects. The efficacy of low doses of nicotinic acid that may produce fewer side effects has not been adequately studied. **OBJECTIVE:** To determine the effects of low-dose nicotinic acid on HDL-C levels in patients with hypoalphalipoproteinemia.

**METHODS:** Forty-four men with low HDL-C levels (< 1.03 mmol/L [ $< 40$  mg/dL]) entered the study. Twenty-four patients otherwise had normal lipid levels, and 20 were moderately hypertriglyceridemic (range of plasma triglyceride levels, 2.82 to 5.64 mmol/L 250 to 500 mg/dL). The trial consisted of 3 phases; each phase lasted 8 weeks. The first phase was diet only (30% fat diet); in the second phase, crystalline nicotinic acid was added at 1.5 g/d; and in the third phase, the dose was increased to 3 g/d.

**RESULTS:** Of the 44 patients who entered the study, 37 completed the low-dose phase (1.5 g/d); the remaining patients were withdrawn because of side effects to nicotinic acid. Four other patients who completed the low-dose phase were excluded from the higher dose phase because of side effects that developed when they were receiving the low dose. Ten other patients withdrew during the high-dose phase because of side effects. In both groups, responses to nicotinic acid therapy tended to be dose-dependent. For both groups, the higher dose generally produced a greater reduction in apolipoprotein B-containing lipoproteins and a

greater rise in HDL-C levels. However, for both groups, the low dose of nicotinic acid gave an average 20% increase in HDL-C levels.

**CONCLUSIONS:** A low dose (1.5 g/d) of crystalline nicotinic acid causes an average 20% increase in HDL-C levels and significantly lowers triglyceride levels in both normolipidemic and hyperlipidemic patients with low HDL-C levels. Although the changes induced by this dose are less than those that can be achieved by a higher dose, the lower dose is better tolerated. Nicotinic acid may be useful in combined drug therapy for secondary prevention of coronary heart disease, and if higher doses cannot be tolerated, use of a lower dose should still be useful for producing a moderate rise in HDL-C levels in patients with hypoalphalipoproteinemia.

### **Clinical trial experience with extended-release niacin (Niaspan): dose-escalation study.**

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Am J Cardiol 1998 Dec 17;82(12A):35U-38U; discussion 39U-41U

Niacin is a useful lipid-modifying drug because it (1) decreases low-density lipoprotein (LDL) cholesterol, total cholesterol, triglycerides, and lipoprotein(a), and (2) raises high-density lipoprotein (HDL) cholesterol. Its use tends to be limited by side effects and inconvenient dosing regimens. The availability of an extended-release preparation (Niaspan-which has safety and efficacy similar to immediate-release niacin but which can be given once a day) provides an opportunity to increase the use of this effective lipid-modifying agent. To study the safety and efficacy of escalating doses of extended-release niacin, hyperlipidemic patients were randomly assigned to placebo or Niaspan. A forced dose-titration was done with the dosage increasing by 500 mg every 4 weeks to a maximum of 3,000 mg/day. Niaspan showed dose-related changes in total, LDL, and HDL cholesterol levels, triglycerides, cholesterol/HDL ratio, and lipoprotein(a). At a dosage of 2,000 mg/day, total cholesterol decreased by 12.1%, LDL cholesterol by 16.7%, triglycerides by 34.5%, and lipoprotein(a) by 23.6%; HDL cholesterol increased by 25.8%. Flushing was the most commonly reported side effect; flushing episodes tended to decrease with time despite an increasing dose of niacin. Of the reported side effects, only pruritus and rash were significantly different between the 2 groups. Aspartate aminotransferase, lactate dehydrogenase, and uric acid increased in a dose-dependent fashion, but fasting blood sugar increased by about 5% across most dosages. Two subjects had aspartate aminotransferase levels greater than twice the upper limit of normal, but there were no subjects in whom transaminases increased to 3 times the upper limit of normal. Women tended to have a greater LDL cholesterol response to the medication and also experienced more side effects, especially at higher dosages. Thus, the use of lower dosages of niacin may be desirable in women. The results of this dose-escalation study show beneficial effects of Niaspan on the entire lipid

profile. At the maximum recommended dosage of 2,000 mg/day, all lipid and lipoprotein levels changed in desirable directions. Side effects (other than flushing) and blood chemistries were comparable to those seen with immediate-release niacin.

### **Vitamin E and atherosclerosis.**

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J Nutr 1998 Oct;128(10):1593-6

Vitamin E was advocated as an effective treatment for heart disease by Dr. Even Shute of London, Ontario more than 50 years ago. His pioneering claims, which were unacceptable to the medical community at large, have been confirmed by recent findings from epidemiologic studies and clinical trials. This review integrates our current knowledge of atherogenesis with the biological functions of vitamin E. The response-to-injury hypothesis explains atherosclerosis as a chronic inflammatory response to injury of the endothelium, which leads to complex cellular and molecular interactions among cells derived from the endothelium, smooth muscle and several blood cell components. Inflammatory and other stimuli trigger an overproduction of free radicals, which promote peroxidation of lipids in LDL trapped in the subendothelial space. Products of LDL oxidation are bioactive, and they induce endothelial expression and secretion of cytokines, growth factors and several cell surface adhesion molecules. The last-mentioned are capable of recruiting circulating monocytes and T lymphocytes into the intima where monocytes are differentiated into macrophages, the precursor of foam cells. In response to the growth factors and cytokines, smooth muscle cells proliferate in the intima, resulting in the narrowing of the lumen. Oxidized LDL can also inhibit endothelial production of prostacyclin and nitric oxide, two potent autacoids that are vasodilators and inhibitors of platelet aggregation. Evidence is presented that vitamin E is protective against the development of atherosclerosis. Vitamin E enrichment has been shown to retard LDL oxidation, inhibit the proliferation of smooth muscle cells, inhibit platelet adhesion and aggregation, inhibit the expression and function of adhesion molecules, attenuate the synthesis of leukotrienes and potentiate the release of prostacyclin through up-regulating the expression of cytosolic phospholipase A2 and cyclooxygenase. Collectively, these biological functions of vitamin E may account for its protection against the development of atherosclerosis.

### **Dynamics of vitamin E action against LDL oxidation.**

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Free Radic Res 1998 Jun;28(6):561-72

Vitamin E acts as an important antioxidant against oxidative modification of low density lipoprotein (LDL) which is accepted as an initial event in the pathogenesis of atherosclerosis. In spite of the numerous studies and reports, the action and role of vitamin E have not been fully elucidated yet. In this brief overview, the dynamics of action of vitamin E as an antioxidant have been discussed and it is emphasized that the total antioxidant potency is determined by the relative importance of many competing reactions which is determined by the reactivities and concentrations of substrates, radicals and antioxidant and by physical factors of the environment.

**Cost-effectiveness of vitamin E therapy in the treatment of patients with angiographically proven coronary narrowing (CHAOS trial). Cambridge Heart Antioxidant Study.**

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Am J Cardiol 1998 Aug 15;82(4):414-7

Epidemiologic studies have suggested that vitamin E (alpha-tocopherol) may play a preventive role in reducing the incidence of atherosclerosis. The aim of this paper was to conduct a cost-effectiveness analysis of vitamin E supplementation in patients with coronary artery disease using data from the Cambridge Heart Antioxidant Study (CHAOS). The study compared cost-effectiveness in the context of Australian and United States (US) health care utilization. The main clinical outcome used in the economic evaluation was the incidence of acute myocardial infarction (AMI) which was nonfatal. Utilization of health care resources was estimated by conducting a survey of Australian clinicians and published Australian and US cost data. Cost savings of \$127 (A\$181) and \$578/patient randomized to vitamin E therapy compared with patients receiving placebo were found for Australian and US settings, respectively. Savings in the vitamin E group were due primarily to reduction in hospital admissions for AMI. This occurred because the vitamin E group had a 4.4% lower absolute risk of AMI than did the placebo group. Less than 10% of health care costs in the Australian evaluation was due to vitamin E (\$150 [A\$214/patient]). Our economic evaluation indicates that vitamin E therapy in patients with angiographically proven atherosclerosis is cost-effective in the Australian and US settings.

**Antioxidant vitamin intake and coronary mortality in a longitudinal population study.**

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Am J Epidemiol 1994 Jun 15;139(12):1180-9

Oxidation of lipoproteins is hypothesized to promote atherosclerosis and, thus, a high intake of antioxidant nutrients may protect against coronary heart disease. The relation between the intakes of dietary carotene, vitamin C, and vitamin E and the subsequent coronary mortality was studied in a cohort of 5,133 Finnish men and women aged 30-69 years and initially free from heart disease. Food consumption was estimated by the dietary history method covering the total habitual diet during the previous year. Altogether, 244 new fatal coronary heart disease cases occurred during a mean follow-up of 14 years beginning in 1966-1972. An inverse association was observed between dietary vitamin E intake and coronary mortality in both men and women with relative risks of 0.68 ( $p$  for trend = 0.01) and 0.35 ( $p$  for trend < 0.01), respectively, between the highest and lowest tertiles of the intake. Similar associations were observed for the dietary intake of vitamin C and carotenoids among women and for the intake of important food sources of these micronutrients, i.e., of vegetables and fruits, among both men and women. The associations were not attributable to confounding by major nondietary risk factors of coronary heart disease, i.e., age, smoking, serum cholesterol, hypertension, or relative weight. The results support the hypothesis that antioxidant vitamins protect against coronary heart disease, but it cannot be excluded that foods rich in these micronutrients also contain other constituents that provide the protection.

### **Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease?**

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Free Radic Res 1997 Nov;27(5):511-32  
Corrected and republished article originally printed in Free Radic Res 1997 Jun;26(6):565-83

Recent research about the role of free radical derivatives of oxygen and nitrogen in biological systems has highlighted the possibility that antioxidants, such as vitamin E, that prevent these processes in vitro may be capable of carrying out a similar function in living organisms in vivo. There is increasing evidence that free radical reactions are involved in the early stages, or sometimes later on, in the development of human diseases, and it is therefore of particular interest to inquire whether vitamin E and other antioxidants, which are found in the human diets, may be capable of lowering the incidence of these diseases. Put simply, the proposition is that by improving human diets by increasing the quantity in them of antioxidants, it might be possible to reduce the incidence of a number of degenerative diseases. Of particular significance to these considerations is the likely role of the primary fat-soluble dietary antioxidant vitamin E in the prevention of degenerative diseases such as arteriosclerosis, which is frequently

the cause of consequent heart attacks or stroke, and prevention of certain forms of cancer, as well as several other diseases. Substantial evidence for this proposition now exists, and this review is an attempt to give a brief account of the present position. Two kinds of evidence exist; on the one hand there is very substantial basic science evidence which indicates an involvement of free radical events, and a preventive role for vitamin E, in the development of human disease processes. On the other hand, there is also a large body of human epidemiological evidence which suggests that incidence of these diseases is lowered in populations having a high level of antioxidants, such as vitamin E, in their diet, or who have taken steps to enhance their level of intake of the vitamin by taking dietary supplements. There is also some evidence which suggests that intervention with dietary supplements of vitamin E can result in a lowered risk of disease, in particular of cardiovascular disease, which is a major killer disease among the developed nations of the world. The intense interest in this subject recently has as its objective the possibility that, by making some simple alterations to dietary lifestyle, or by enhancing the intake of vitamin E by fortification of foods, or by dietary supplements, it may be possible to reduce substantially the risk of a large amount of common, highly disabling human disease. By this simple means, therefore it may be possible to improve substantially the quality of human life, in particular for people of advancing years.

### **Probucol and multivitamins in the prevention of restenosis after coronary angioplasty. Multivitamins and Probucol Study Group.**

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N Engl J Med 1997 Aug 7;337(6):365-72

Comment in: N Engl J Med 1997 Dec 25;337(26):1918; discussion 1919

**BACKGROUND:** Oxidizing metabolites generated at the site of coronary angioplasty can induce chain reactions that may lead to restenosis. Antioxidants may counter oxidative stress and modify neointimal formation and vascular remodeling. Experimental data and small clinical studies have suggested that antioxidants may prevent restenosis after angioplasty. In a double-blind, randomized trial, we studied whether drugs with antioxidant properties decrease the incidence and severity of restenosis after angioplasty.

**METHODS:** One month before angioplasty, 317 patients were randomly assigned to receive one of four treatments: placebo, probucol (500 mg), multivitamins (30,000 IU of beta carotene, 500 mg of vitamin C, and 700 IU of vitamin E), or both probucol and multivitamins—all given twice daily. Patients were treated for four weeks before and six months after angioplasty. Patients received an extra 1000 mg of probucol, 2000 IU of vitamin E, both probucol and vitamin E, or placebo 12 hours before angioplasty, according to their treatment assignments. Base-line and follow-up angiograms were interpreted by blinded investigators using a quantitative approach.

**RESULTS:** The mean ( $\pm$ SD) reduction in luminal diameter six months after angioplasty was 0.12  $\pm$  0.41 mm in the probucol group, 0.22  $\pm$  0.46 mm in the combined-treatment group, 0.33  $\pm$  0.51 in the multivitamin group, and 0.38  $\pm$  0.50 mm in the placebo group ( $P = 0.006$  for those receiving vs. those not receiving probucol, and  $P = 0.70$  for those receiving vs. those not receiving vitamins. Restenosis rates per segment were 20.7 percent in the probucol group, 28.9 percent in the combined-treatment group, 40.3 percent in the multivitamin group, and 38.9 percent in the placebo group ( $P = 0.003$  for probucol vs. no probucol). The rates of repeat angioplasty were 11.2 percent, 16.2 percent, 24.4 percent, and 26.6 percent, respectively ( $P = 0.009$  for probucol vs. no probucol).

**CONCLUSIONS:** The antioxidant probucol is effective in reducing the rate of restenosis after balloon coronary angioplasty.

**Effects of intravenous perilla oil emulsion on nutritional status, polyunsaturated fatty acid composition of tissue phospholipids, and thromboxane A2 production in streptozotocin-induced diabetic rats.**

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Nutrition 1995 Sep-Oct;11(5):450-5

The effects of a perilla oil (PO) emulsion rich in alpha-linolenic acid, administered by intravenous infusion, on nutritional status, fatty acid composition, and thromboxane A2 production were compared with those of a soybean oil (SO) emulsion in streptozotocin-induced diabetic rats given a fat-free diet for 7 days. The PO emulsion improved body weight gain and nitrogen balance compared with the SO emulsion and reduced thromboxane A2 production by platelets. The PO emulsion also increased the proportion of eicosapentaenoic acid, but decreased that of arachidonic acid, in liver and serum phospholipids. Plasma insulin concentrations and blood biochemical indices were similar in the two groups. An intravenously infused PO emulsion effectively reduces thromboxane A2 production through changes in the fatty acid composition of liver and serum phospholipids, as with oral administration, and improves the nutritional status of diabetic rats.

**Homocysteine metabolism and risk of myocardial infarction: relation with vitamins B6, B12, and folate.**

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Am J Epidemiol 1996 May 1;143(9):845-59

Elevated plasma homocyst(e)ine levels are an independent risk factor for vascular disease. In a case-control study, the authors studied the associations of fasting plasma homocyst(e)ine and vitamins, which are important cofactors in homocysteine metabolism, with the risk of myocardial infarction. The cases were 130 Boston area patients hospitalized with a first myocardial infarction and 118 population controls, less than 76 years of age, enrolled in 1982 and 1983. Dietary intakes of vitamins B6, B12, and folate were estimated from a food frequency questionnaire. After adjusting for sex and age, the authors found that the geometric mean plasma homocyst(e)ine level was 11% higher in cases compared with controls ( $p = 0.006$ ). There was no clear excess of cases with extremely elevated levels. The age- and sex-adjusted odds ratio for each 3- $\mu\text{mol/liter}$  (approximately 1 standard deviation) increase in plasma homocyst(e)ine was 1.35 (95% confidence interval 1.05-1.75;  $p$  trend = 0/007). After further control for several risk factors, the odds ratio was not affected, but the confidence interval was wider and the  $p$  value for trend was less significant. Dietary and plasma levels of vitamin B6 and folate were lower in cases than in controls, and these vitamins were inversely associated with the risk of myocardial infarction, independently of other potential risk factors. Vitamin B12 showed no clear association with myocardial infarction, although methylmalonic acid levels were significantly higher in cases. Comparing the mean levels of several homocysteine metabolites among cases and controls, the authors found that impairment of remethylation of homocyst(e)ine (dependent of folate and vitamin B12 rather than on vitamin B6-dependent transsulfuration) was the predominant cause of high homocyst(e)ine levels in cases. Accordingly, plasma folate and, to a lesser extent, plasma vitamin B12, but not vitamin B6, correlated inversely with plasma homocyst(e)ine, even for concentrations at the high end of normal values. These data provide further evidence that plasma homocyst(e)ine is an independent risk factor for myocardial infarction. In this population, folate was the most important determinant of plasma homocyst(e)ine, even in subjects with apparently adequate nutritional status of this vitamin.

### **Homocysteine and cardiovascular disease.**

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Annu Rev Med 1998;49:31-62

An elevated level of total homocysteine (tHcy) in blood, denoted hyperhomocysteinemia, is emerging as a prevalent and strong risk factor for atherosclerotic vascular disease in the coronary, cerebral, and peripheral vessels, and for arterial and venous thromboembolism. The basis for these conclusions is data from about 80 clinical and epidemiological studies including more than 10,000 patients. Elevated tHcy confers a graded risk with no threshold, is independent of but may enhance the effect of the conventional risk factors, and seems to be a particularly strong predictor of cardiovascular mortality. Hyperhomocysteinemia is attributed to commonly occurring genetic and acquired

factors including deficiencies of folate and vitamin B12. Supplementation with B-vitamins, in particular with folic acid, is an efficient, safe, and inexpensive means to reduce an elevated tHcy level. Studies are now in progress to establish whether such therapy will reduce cardiovascular risk.

### **The antiatherosclerotic effect of *Allium sativum*.**

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*Atherosclerosis* 1999 May;144(1):237-49

In a randomized, double-blind, placebo-controlled clinical trial, the plaque volumes in both carotid and femoral arteries of 152 probationers were determined by B-mode ultrasound. Continuous intake of high-dose garlic powder dragees reduced significantly the increase in arteriosclerotic plaque volume by 5-18% or even effected a slight regression within the observational period of 48 months. Also the age-dependent representation of the plaque volume shows an increase between 50 and 80 years that is diminished under garlic treatment by 6-13% related to 4 years. It seems even more important that with garlic application the plaque volume in the whole collective remained practically constant within the age-span of 50-80 years. These results substantiated that not only a preventive but possibly also a curative role in arteriosclerosis therapy (plaque regression) may be ascribed to garlic remedies.

### **Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgus monkeys.**

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*Metabolism (United States)* Jun 1997, 46 (6) p698-705

Estrogen replacement therapy (ERT) decreases the progression of coronary artery atherosclerosis in monkeys. Dietary soy protein also retards the progression of atherosclerosis relative to animal proteins such as casein. Soy protein contains weakly estrogenic compounds called isoflavones or phytoestrogens that may be responsible for the cardioprotective effects. This study was designed as a 2 x 2 factorial to determine the magnitude of soy protein's effects on cardiovascular risk factors relative to casein and lactalbumin, with or without estradiol treatment. Ovariectomized female monkeys were randomized to four treatment groups based on past dietary cholesterol consumption, their origin, and past reproductive history, and studied for 7 months. The animals were divided into (1) a group fed

casein and lactalbumin as the protein source (n = 14), (2) a group fed casein and lactalbumin as the protein source plus 17 beta-estradiol (E2) (n = 13), (3) a group fed soybean protein isolate as the protein source (n = 11), and (4) a group fed soybean protein isolate as the protein source plus E2 (n = 10). Soy protein compared with casein consumption resulted in a significant improvement in plasma lipid and lipoprotein concentrations, a significant improvement in insulin sensitivity and glucose effectiveness as determined by minimal-model analyses, and a decrease in arterial lipid peroxidation. E2-treated monkeys had a significant reduction in fasting insulin levels and insulin to glucose ratios, total body weight, and amounts of abdominal fat, and had smaller low-density lipoprotein (LDL) particles. In addition, E2 treatment resulted in a significant reduction (P = .001) in aortic cholesteryl ester content. A similar trend (P = .14) was found for soy protein compared with casein. There also was a significant interaction (P = .02) with soy and E2, such that animals consuming soy protein +E2 had the least arterial cholesteryl ester content. These results suggest that both ERT and dietary soybean protein have beneficial effects on cardiovascular risk factors. Interestingly, the two treatments affected different risk factors and together resulted in the greatest reduction in arterial cholesterol content. Further studies are needed to determine the active component of the soy protein and to assess its long-term effects on the cardiovascular system and other organ systems (such as the bones and reproductive system).

### **Hyperhomocysteinemia and venous thromboembolic disease.**

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Haematologica (Italy) Mar-Apr 1997, 82 (2) p211-9

**BACKGROUND AND OBJECTIVE:** In spite of the large number of reports showing that hyperhomocysteinemia (HHcy) is an independent risk factor for atherosclerosis and arterial occlusive disease, this metabolite of the methionine pathway is measured in relatively few laboratories and its importance is not fully appreciated. Recent data strongly suggest that mild HHcy is also involved in the pathogenesis of venous thromboembolic disease. The aim of this paper is to analyze the most recent advances in this field.

**EVIDENCE AND INFORMATION SOURCES:** The material examined in the present review includes articles and abstracts published in journals covered by the Science Citation Index and Medline. In addition the authors of the present article have been working in the field of mild HHcy as cause of venous thromboembolic disease.

**STATE OF ART AND PERSPECTIVES:** The studies examined provide very strong evidence supporting the role of moderate HHcy in the development of premature and/or recurrent venous thromboembolic disease. High plasma homocysteine levels are also a risk factor for deep vein thrombosis in the general population. Folic acid fortification of food has been proposed as a major tool for

reducing coronary artery disease mortality in the United States. Vitamin supplementation may also reduce recurrence of venous thromboembolic disease in patients with HHcy. At the present time, however, the clinical efficacy of this approach has not been tested. In addition, the bulk of evidence indicates that fasting total homocysteine determinations can identify up to 50% of the total population of hyperhomocysteinemic subjects. Patients with isolated methionine intolerance may benefit from vitamin B6 supplementation. Homocysteine-lowering vascular disease prevention trials are urgently needed. Such controlled studies, however, should not focus exclusively on fasting homocysteine determinations and folic acid monotherapy. (127 Refs.)

### **Plasma homocysteine as a risk factor for vascular disease. The European Concerted Action Project.**

Graham IM; Daly LE; Refsum HM; Robinson K; Brattstrom LE; Ueland PM; Palma-Reis RJ; Boers GH; Sheahan RG; Israelsson B; Uiterwaal CS; Meleady R; McMaster D; Verhoef P; Witteman J; Rubba P; Bellet H; Wautrecht JC; de Valk HW; Sales Luis AC; Parrot-Rouland FM; Tan KS; Higgins I; Garcon D; Andria G ; et al

Department of Cardiology, Adelaide Hospital, Trinity College, Dublin, Ireland.  
JAMA (United States) Jun 11 1997, 277 (22) p1775-81

**CONTEXT:** Elevated plasma homocysteine is a known risk factor for atherosclerotic vascular disease, but the strength of the relationship and the interaction of plasma homocysteine with other risk factors are unclear.

**OBJECTIVE:** To establish the magnitude of the vascular disease risk associated with an increased plasma homocysteine level and to examine interaction effects between elevated plasma homocysteine level and conventional risk factors.

**DESIGN:** Case-control study.

**SETTING:** Nineteen centers in 9 European countries.

**PATIENTS:** A total of 750 cases of atherosclerotic vascular disease (cardiac, cerebral, and peripheral) and 800 controls of both sexes younger than 60 years.

**MEASUREMENTS:** Plasma total homocysteine was measured while subjects were fasting and after a standardized methionine-loading test, which involves the administration of 100 mg of methionine per kilogram and stresses the metabolic pathway responsible for the irreversible degradation of homocysteine. Plasma cobalamin, pyridoxal 5'-phosphate, red blood cell folate, serum cholesterol, smoking, and blood pressure were also measured.

**RESULTS:** The relative risk for vascular disease in the top fifth compared with the bottom four fifths of the control fasting total homocysteine distribution was 2.2 (95% confidence interval, 1.6-2.9). Methionine loading identified an

additional 27% of at-risk cases. A dose-response effect was noted between total homocysteine level and risk. The risk was similar to and independent of that of other risk factors, but interaction effects were noted between homocysteine and these risk factors; for both sexes combined, an increased fasting homocysteine level showed a more than multiplicative effect on risk in smokers and in hypertensive subjects. Red blood cell folate, cobalamin, and pyridoxal phosphate, all of which modulate homocysteine metabolism, were inversely related to total homocysteine levels. Compared with nonusers of vitamin supplements, the small number of subjects taking such vitamins appeared to have a substantially lower risk of vascular disease, a proportion of which was attributable to lower plasma homocysteine levels.

**CONCLUSIONS:** An increased plasma total homocysteine level confers an independent risk of vascular disease similar to that of smoking or hyperlipidemia. It powerfully increases the risk associated with smoking and hypertension. It is time to undertake randomized controlled trials of the effect of vitamins that reduce plasma homocysteine levels on vascular disease risk.

**Homocyst(e)ine: an important risk factor for atherosclerotic vascular disease.**

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Curr Opin Lipidol (United States) Feb 1997, 8 (1) p28-34

Homocysteine is an intermediate compound formed during metabolism of methionine. The results of many recent studies have indicated that elevated plasma levels of homocyst(e)ine are associated with increased risk of coronary atherosclerosis, cerebrovascular disease, peripheral vascular disease, and thrombosis. The plasma level of homocyst(e)ine is dependent on genetically regulated levels of essential enzymes and the intake of folic acid, vitamin B6 (pyridoxine), and vitamin B12 (cobalamin). Impaired renal function, increased age, and pharmacologic agents (e.g. nitrous oxide, methotrexate) can contribute to increased levels of homocyst(e)ine. Plausible mechanisms by which homocyst(e)ine might contribute to atherogenesis include promotion of platelet activation and enhanced coagulability, increased smooth muscle cell proliferation, cytotoxicity, induction of endothelial dysfunction, and stimulation of LDL oxidation. Levels of homocysteine can be reduced with pharmacologic doses of folic acid, pyridoxine, vitamin B12, or betaine, but further research is required to determine the efficacy of this intervention in reducing morbidity and mortality associated with atherosclerotic vascular disease.

**[Homocysteine, a risk factor of atherosclerosis]**

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Service de cardiologie B, hopital de la Timone, Marseille.  
Arch Mal Coeur Vaiss (France) Dec 1996, 89 (12) p1667-71

Homocysteine is a sulphurated amino acid which, at high plasma concentrations, predisposes to thrombosis and induces focal arteriosclerosis. These characteristics have been established both in patients with homocystinuria, a genetic disease in which homocysteine accumulates in the blood, and in animals submitted to intravenous infusions of this amino acid. Many recent publications have addressed the problem of whether mild increases in plasma homocysteine predisposed to the development of the usual forms of atherosclerosis. Transverse epidemiological studies have established a correlation between homocysteine levels and atherosclerosis at all its vascular localisations, coronary, carotid and lower limb. Multivariate analysis in several prospective studies have shown plasma homocysteine to be an independent risk factor for cerebrovascular accidents and myocardial infarction. Causes of mild increases in plasma homocysteine are usually dietetic deficiencies in folic acid, vitamin B6 or B12, or genetic by mutation of the methylene-tetrahydrofolate reductase. Renal failure is also associated with a high risk in plasma homocysteine levels. However, the toxicity of homocysteine to the arterial wall at slightly elevated concentration remains speculative.

**Comparison between dietary soybean protein and casein of the inhibiting effect on atherogenesis in the thoracic aorta of hypercholesterolemic (ExHC) rats treated with experimental hypervitamin D.**

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Biosci Biotechnol Biochem (JAPAN) Mar 1997, 61 (3) p514-9

Atherosclerotic lesions of the thoracic aorta were induced in exogenously hypercholesterolemic (ExHC) rats by treating initially with hypervitamin D2 and subsequently feeding on hypercholesterolemic diets for 180 days. Dietary soybean protein, in comparison with casein, substantially decreased the degree of atherosclerotic lesions, which was evaluated by intimal thickening, although with a similar topographical distribution. The casein-fed rats tended to maintain a high concentration of serum cholesterol, particularly in triacylglycerol-rich lipoproteins. The concentrations of apo A-I and TBARS in the serum was comparable between the dietary protein groups. The data suggest that dietary soybean protein, compared to casein, produced lipoproteins which were less atherosclerotic by partitioning cholesterol in the triacylglycerol-poor lipoproteins.

**Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques.**

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Medicine of Wake Forest University, Winston-Salem, North Carolina.  
Fertil Steril (United States) Jan 1997, 67 (1) p148-54

**OBJECTIVE:** To examine the effects of soy phytoestrogens on coronary vascular reactivity in atherosclerotic male and female rhesus monkeys.

**DESIGN:** A prospective, randomized, blinded, controlled study.

**SETTING:** Comparative Medicine Clinical Research Center of an academic medical center.

**PATIENT(S):** Twenty-two young adult rhesus monkeys with pre-existing diet-induced atherosclerosis.

**INTERVENTION(S):** Monkeys were fed soy-based diets for 6 months identical in composition, except that the isoflavones were extracted from one flow-isoflavone) and intact in the other (high-isoflavone). Quantitative coronary angiography was performed at the end of the study period. Females in the low-isoflavone group under went a second angiography after an acute IV dose of genistein.

**MAIN OUTCOME MEASURE(S):** Percent change in diameter of the proximal left circumflex coronary artery in response to intracoronary acetylcholine and nitroglycerin, compared with control diameter.

**RESULT(S):** Arteries from males constricted in response to acetylcholine. Arteries from females in the low-isoflavone group constricted (-6.2% +/- 2.8%, mean +/- SEM), whereas arteries from females in the high-isoflavone group dilated (6.4% +/- 1.2%, mean +/- SEM). Intravenous administration of genistein caused dilation in the previously constricting low-isoflavone females (3.3% +/- 2.8%).

**CONCLUSION(S):** Like mammalian estrogens, dietary soy isoflavones enhance the dilator response to acetylcholine of atherosclerotic arteries in female monkeys.

**Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations.**

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**BACKGROUND:** A high level of total plasma homocysteine is a risk factor for atherosclerosis, which is an important cause of death in renal failure. We evaluated the role of this as a risk factor for vascular complications of end-stage renal disease.

**METHODS AND RESULTS:** Total fasting plasma homocysteine and other risk factors were documented in 176 dialysis patients (97 men, 79 women; mean age, 56.3 +/- 14.8 years). Folate, vitamin B12, and pyridoxal phosphate concentrations were also determined. The prevalence of high total homocysteine values was determined by comparison with a normal reference population, and the risk of associated vascular complications was estimated by multiple logistic regression. Total homocysteine concentration was higher in patients than in the normal population (26.6 +/- 1.5 versus 10.1 +/- 1.7  $\mu\text{mol/L}$ ;  $P < .01$ ). Abnormally high concentrations ( $> 95$ th percentile for control subjects, 16.3  $\mu\text{mol/L}$ ) were seen in 149 patients (85%) with end-stage renal disease ( $P < .001$ ). Patients with a homocysteine concentration in the upper two quintiles ( $> 27.8 \mu\text{mol/L}$ ) had an independent odds ratio of 2.9 (CI, 1.4 to 5.8;  $P = .007$ ) of vascular complications. B vitamin levels were lower in patients with vascular complications than in those without. Vitamin B6 deficiency was more frequent in patients than in the normal reference population (18% versus 2%;  $P < .01$ ).

**CONCLUSIONS:** A high total plasma homocysteine concentration is an independent risk factor for atherosclerotic complications of end-stage renal disease. Such patients may benefit from higher doses of B vitamins than those currently recommended.

### **High dose-B-vitamin treatment of hyperhomocysteinemia in dialysis patients.**

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Kidney Int (United States) Jan 1996, 49 (1) p147-52

Hyperhomocysteinemia, an arteriosclerotic risk factor, persists in 75% of dialysis patients despite routine low dose supplementation with the B-vitamin co-factors/substrates for homocysteine (Hcy) metabolism, and normal or supernormal plasma status of these vitamins (Atherosclerosis 114:93, 1995). We conducted a placebo-controlled eight-week trial of the effect on plasma homocysteine of adding supraphysiologic dose folic acid (15 mg/day), B-6 (100 mg/day), and B-12 (1 mg/day) to the usual daily dosing of 1 mg folic acid, 10 mg B-6, and 12 micrograms B-12, in 27 hyperhomocysteinemic dialysis patients. Total plasma homocysteine was measured at baseline, and after four and eight weeks. Blinded analyses revealed no evidence of toxicity in the group randomized

to supraphysiologic dose B-vitamin supplementation. Plasma homocysteine was significantly reduced after both four weeks (-29.8% vs. -2.0%; P = 0.0024) and eight weeks (-25.8% vs. +0.6%; P = 0.0009) of active versus placebo treatment. Also, 5 of 15 treated versus 0 of 12 placebo group patients had their plasma Hcy reduced to within the normative range (< 15  $\mu\text{mol/liter}$ ). Supraphysiologic doses of B-vitamins may be required to correct hyperhomocysteinemia in dialysis patients.

### **Measurement of the ratio between the reduced and oxidized forms of coenzyme Q10 in human plasma as a possible marker of oxidative stress.**

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J Lipid Res (United States) Jan 1996, 37 (1) p67-75

It has been postulated that lipid peroxidation plays a crucial role in the pathogenesis of atherosclerosis. As CoQ10H<sub>2</sub> (reduced form of coenzyme Q10) is easily oxidized to CoQ10 (oxidized form of coenzyme Q10), it has been proposed that the CoQ10H<sub>2</sub>/CoQ10 ratio may be used as a possible marker of in vivo oxidative stress. However, sample preparation has an important effect on the redox status of coenzyme Q10 due to the extreme sensitivity of CoQ10H<sub>2</sub> towards oxidation. We now report a rapid, simple isocratic HPLC procedure for the determination of CoQ10H<sub>2</sub> and CoQ10 in plasma isopropanol extracts, and we used this method to investigate conditions by which the CoQ10H<sub>2</sub>/CoQ10 ratio can be reliably measured. Our results indicate that CoQ10H<sub>2</sub> is unstable in whole blood, plasma, and isopropanol extracts; subsequently the CoQ10H<sub>2</sub>/CoQ10 ratio changes considerably soon after a blood sample has been obtained. The time period since blood sampling and HPLC analysis, as well as the sample pretreatment procedure, are two factors that have a profound effect on the pre-analytical variation in the determination of the CoQ10H<sub>2</sub>/CoQ10 ratio. If these two factors are properly controlled, the CoQ10H<sub>2</sub>/CoQ10 ratio may be a sensitive and practical way to measure in vivo oxidative stress. Furthermore, this indicator is independent from plasma total cholesterol concentrations, implying that groups who differ with respect to cholesterol levels may be compared directly.

### **Hyperhomocysteinemia induced by folic acid deficiency and methionine load-applications of a modified HPLC method.**

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Clin Chim Acta (Netherlands) Aug 15 1996, 252 (1) p83-93

The increasing possibility that homocysteine might be involved in atherosclerosis in non-homocysteinuric subjects has required the measurement of low concentrations of this aminothiols in biological samples. The procedure described here represents an improvement of different HPLC methods. We utilized an isocratic HPLC system with fluorescence detection of plasma total homocysteine derivatized after reaction with ammonium 7-fluoro-benzo-2-oxa-1,3-diazole-4-sulphonate. With the help of the rapidly eluting internal standard N-acetylcysteine, the method ensures very good recovery (approximately 100%), reproducibility and precision (within-assay 2.31%; day-to-day: 2.8%) in the physiological concentration range. This procedure allowed us to validate various animal models of hyperhomocysteinemia such as dietary folic acid deficiency in rat and acute methionine loads in rat and hamster. Using this method, we also confirmed that men have higher plasma total homocysteine levels than women. Due to its simplicity and reliability, our procedure is suitable for routine analysis of total homocysteine and other aminothiols (cysteine, cysteinyl-glycine and glutathione) in biological samples, as required in clinical and research laboratories.

### **Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys.**

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J Nutr (United States) Jan 1996, 126 (1) p43-50

Although the beneficial effects of dietary soybean protein compared with animal proteins on plasma lipids, lipoproteins and atherosclerosis have been known for about 50 years, it has been uncertain whether these effects are due to its amino acid concentrations or other components in soybeans. To assess the effect of soybean protein's alcohol-extractable components (including the isoflavonic phytoestrogens genistein and daidzein) on plasma lipid and lipoprotein concentrations and to establish its lack of effect on the reproductive system, we fed 27 peripubertal male and female rhesus monkeys moderately atherogenic diets in which the source of dietary protein was a soy isolate (20% by weight), either containing phytoestrogens (also termed isoflavones) or with the phytoestrogens removed by alcohol extraction. The study was a crossover design with each period lasting for 6 mo. The phytoestrogen-intact soy protein (compared with the alcohol-extracted soy protein) had favorable effects on plasma lipid and lipoprotein concentrations, specifically by significantly reducing LDL+VLDL cholesterol concentrations in both males and females (approximately 30-40% lower), significantly increasing high density lipoprotein cholesterol (HDL) concentrations for females (approximately 15% higher) and significantly lowering total plasma cholesterol (TPC):HDL ratios (approximately 20% lower for males and 50% lower for females). The phytoestrogens had no adverse effects on the reproductive systems of either the males or females, as evaluated by reproductive hormone concentrations and organ weights at necropsy. Thus, the isoflavones in

soy protein improve cardiovascular disease risk factors without apparent deleterious effects on the reproductive system of peripubertal rhesus monkeys.

### **Therapeutic actions of garlic constituents.**

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Med Res Rev (United States) Jan 1996, 16 (1) p111-24

Most studies on garlic during the past 15 years have been primarily in the fields of cardiovascular and cancer research. Cardiovascular studies have been mainly related to atherosclerosis, where effects were examined on serum cholesterol, LDL, HDL, and triglycerides. Although the studies were not consistent in relation to the dosage, standardization of garlic preparations, and period of treatment, most findings suggest that garlic decreases cholesterol and triglycerides levels in patients with increased levels of these lipids. Lowering of serum lipids by garlic ingestion may decrease the atherosclerosis process. The other major beneficial effect of garlic is due to its antithrombotic actions. This field of garlic research has been extensively studied. Garlic extracts and several garlic constituents demonstrate significant antithrombotic actions both in vitro and in vivo systems. Allicin and adenosine are the most potent antiplatelet constituents of garlic because of their in vitro effects. Since both allicin and adenosine are rapidly metabolized in human blood and other tissues, it is doubtful that these compounds contribute to any antithrombotic actions in the body. In addition, ajoene also seems not to be an active antiplatelet principle, because it is not naturally present in garlic, garlic powders, or other commercial garlic preparations. Only a small amount of ajoene can be found in garlic oil-macerates; however, ajoene is being developed as a drug for treatment of thromboembolic disorders. Recent findings on the identification of potent enzyme inhibiting activities of adenosine deaminase and cyclic AMP phosphodiesterase in garlic extracts are interesting, and may have a significant role in the pharmacological actions in the body. Presence of such enzyme inhibitors in garlic may perhaps explain several clinical effects in the body, including the antithrombotic, vasodilatory, and anticancer actions. Epidemiological studies have suggested that garlic plays a significant role in the reduction of deaths caused by malignant diseases. This had led many investigators to examine garlic and garlic constituents for their antitumor and cytotoxic actions both in vitro and in laboratory animals. The data from these investigations suggest that garlic contains several potentially important agents that possess antitumor and anticarcinogenic properties. In summary, the epidemiological, clinical, and laboratory data have proved that garlic contains many biologically and pharmacologically important compounds, which are beneficial to human health from cardiovascular, neoplastic, and several other diseases. Numerous studies are in progress all over the world to develop effective and odorless garlic preparations, as well as to isolate the active principles that may be therapeutically useful. (132 Refs.)

### **Prevention of preatheromatous lesions in sand rats by treatment with a nutritional supplement.**

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Arzneimittelforschung (Germany) Jun 1996, 46 (6) p610-4

Sand rats fed a hypercholesterolaemic diet containing 0.01% of the anti-thyroid agent 2-mercapto-1-imidazole develop preatheromatous lesions similar to those found in humans, in addition to obesity and insulin resistance. The effects of a nutritional supplement rich in essential fatty acids and garlic extract (Arterodiet) on the appearance and evolution of the lesions were studied. Treatment with this nutritional supplement significantly decreased circulating triglycerides and low-density lipoprotein (LDL)-cholesterol levels but did not alter plasma insulin or glucose levels. Intra-arterial cholesterol levels were also decreased by the treatment which resulted in a normalisation of the atherosclerotic lesions in these animals.

### **Evaluation of hydroxyl radical-scavenging property of garlic.**

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Mol Cell Biochem (Netherlands) Jan 12 1996, 154 (1) p55-63

Garlic has been reported to provide protection against hypercholesterolemic atherosclerosis and ischemia-reperfusion-induced arrhythmias and infarction. Oxygen free radicals (OFRs) have been implicated as causative factors in these diseases and antioxidants have been shown to be effective against these conditions. The effectiveness of garlic in these disease states could be due to its ability to scavenge OFRs. However, the OFR-scavenging activity of garlic is not known. Also it is not known if its activity is affected by cooking. We therefore investigated, using high pressure liquid chromatography, the ability of garlic extract (heated or unheated) to scavenge exogenously generated hydroxyl radical (.OH). .OH was generated by photolysis of H<sub>2</sub>O<sub>2</sub> (1.2-10 μmoles/ml) with ultraviolet (UV) light and was trapped with salicylic acid (500 nmoles/ml). H<sub>2</sub>O<sub>2</sub> produced .OH in a concentration-dependent manner as estimated by .OH adduct products 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA. Garlic extract (5-100 microliters/ml) produced an inhibition (30-100%) of 2,3-DHBA and 2,5-DHBA generated by photolysis of H<sub>2</sub>O<sub>2</sub> (5.00 μmoles/ml) in a concentration-dependent manner. Its activity is reduced by 10% approximately when heated to 100 degrees C for 20, 40 or 60 min. The extent of reduction in activity was similar for the three heating periods. Garlic extract prevented the .OH-induced formation of malondialdehyde in the rabbit liver homogenate in a concentration-dependent

manner. It alone did not affect the MDA levels in the absence of .OH. These results indicate that garlic extract is a powerful scavenger of .OH and that heating reduces its activity slightly.

### **[Hyperhomocysteinemia]**

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Cas Lek Cesk (Czech Republic) May 2 1996, 135 (9) p266-9

Similarly as in other inborn metabolic diseases the cause of hyperhomocysteinemia are interactions between genetically conditioned changes most frequently due to reduced cystathionine-beta synthase activities and negative factors of the external environment. Negative environmental factors include above all a high dietary animal protein consumption which is the main methionine donor and a low intake of protein of plant origin. Another negative factor is a low intake of foods of plant origin. Fruits and vegetables are among others important sources of folic acid and pyridoxine. Substitution therapy with vitamin preparations is essential in homozygotes and in high risk heterozygotes of cystathionine beta-synthase. This treatment is also necessary during the periconception period in hyperhomocysteinemic fertile women to reduce the risk of neurotubal defects in their future children. So far investigations are lacking which would provide evidence of a reduced risk of ischaemic heart disease and other cardiovascular diseases in isolated treatment of mildly elevated levels of plasma homocysteine. To elucidate the part played by hyperhomocysteinemia in hastening of the atherogenetic process further studies are essential, focused on the interaction of elevated homocysteine plasma levels, dyslipoproteinaemias, hyperfibrinogenaemia and other metabolic indicators in this process. (31 Refs.)

### **[Homocysteine, a less well-known risk factor in cardiac and vascular diseases]**

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II. interni klinika LF UK a FN Plzen.

Cas Lek Cesk (Czech Republic) May 2 1996, 135 (9) p263-5

Hyperhomocyst(e)mia (Hcy) negatively influences vascular endothelium and coagulation factors. Association of Hcy with premature arteriosclerosis (rather than atherosclerosis), stroke, myocardial infarction and peripheral arterial and venous disease was proved in clinical and epidemiological studies, even as the association with conventional risk factors like age, male sex, smoking, hypertension and hypercholesterolemia. Vitamin substitution of folates, vitamin B6 and B12 decreases Hcy blood levels, however definite evidence is still lacking, whether it results in lower incidence and mortality from cardiovascular diseases. Therefore clinical and epidemiological studies are necessary. Before the

grant-application we proved in a pilot study significantly higher Hcy levels in 97 patients with manifest ischaemic heart disease than in 37 controls.

**Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure.**

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Miner Electrolyte Metab (Switzerland) 1996, 22 (1-3) p106-9

Moderate hyperhomocysteinemia, a risk factor for premature atherosclerosis, is present in chronic uremic patients. We prospectively evaluated the effects of sequential supplementation with pyridoxine (70 mg/day) and folic acid (10 mg/day) for two 3-month periods in 37 nondialyzed patients (29 males) with creatinine clearance (Ccr) ranging from 10 to 80 ml/min, whose plasma vitamin B12 and folate level was in the normal range. Mean (+/- SD) baseline plasma total homocysteine (Hcy) was 14.9 +/- 5.2, 16.5 +/- 5.1 and 26.1 +/- 12.1 mumol/l (upper limit in 45 healthy controls 14.1 mumol/l) in patients with CCr 40-80, 20-40 and < 20 ml/min, respectively. Following pyridoxine Hcy did not significantly decrease whereas following folic acid Hcy decreased significantly to 9.9 +/- 2.9 (-33% vs. baseline), 10.3 +/- 3.4 (-37%) and 15.4 +/- 5.5 (-40%), respectively (Student's paired t test,  $p < 0.001$ ) in the 3 groups. We conclude that folate (but not pyridoxine) pharmacologic supplementation is effective in lowering elevated plasma Hcy in chronic renal failure patients, thus suggesting that enhancing the Hcy remethylation pathway may overcome hyperhomocysteinemia in such patients. In view of the potential atherogenic effects of hyperhomocysteinemia, long-term folate supplementation should be considered in uremic patients.

**Fish oil supplementation in patients with heterozygous familial hypercholesterolemia.**

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Recenti Prog Med (Italy) Mar 1996, 87 (3) p102-5

Familial hypercholesterolemia is associated with premature coronary heart disease. In patients with familial hypercholesterolemia, monotherapy with hydroxymethylglutaryl coenzyme A reductase inhibitors rarely achieves the goal of desirable low-density lipoprotein levels. Epidemiological studies suggest that populations with a high dietary intake of marine n3 fatty acids are protected against coronary heart disease. Hepatic synthesis and secretion of very low density lipoproteins are reduced during fish oil supplementation while other effects on lipid and lipoprotein metabolism are controversial. Fourteen patients affected by familial heterozygous hypercholesterolemia on chronic treatment with simvastatin were enrolled in a double blind, placebo controlled, randomized

crossover trial that evaluated the effect of fish oil ethyl ester (Esapent, 5.1 g/day) on lipid and lipoprotein serum concentrations. Total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, apoprotein B, apoprotein AI, lipoprotein (a) did not show any significant variation during the four week treatment period with fish oil ethyl ester. The present data suggest that the possible favourable influence of fish oil on the progression of atherosclerosis in these high-risk patients might involve mechanisms which are different from lipid metabolism.

### **Homocysteine and coronary atherosclerosis.**

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J Am Coll Cardiol (United States) Mar 1 1996, 27 (3) p517-27

Homocysteine is increasingly recognized as a risk factor for coronary artery disease. An understanding of its metabolism and of the importance of vitamins B6 and B12 and folate as well as enzyme levels in its regulation will aid the development of therapeutic strategies that, by lowering circulating concentrations, may also lower risk. Possible mechanisms by which elevated homocysteine levels lead to the development and progression of vascular disease include effects on platelets, clotting factors and endothelium. This review presents the clinical and basic scientific evidence supporting the risk and mechanisms of vascular disease associated with elevated homocysteine concentrations as well as the results of preliminary therapeutic trials.

### **Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. A case-control study. The ARIC Study Investigators. Atherosclerosis Risk in Communities.**

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Arterioscler Thromb Vasc Biol (United States) Jun 1997, 17 (6) p1171-7

Oxidative modification of LDL is believed to be a crucial step in atherosclerosis. Thus, antioxidant vitamins may have a role in the prevention of coronary disease. We examined the cross-sectional association of serum vitamin levels, the susceptibility of LDL to hemin-induced oxidation (lag phase to conjugated diene formation), and the malondialdehyde-LDL (MDA-LDL) to native LDL radioactivity binding ratio with carotid intima-media thickness (IMT), a measure of asymptomatic early atherosclerosis. The participants in this observational study were 231 asymptomatic age-, sex-, race-, and field center-matched case-control

pairs selected from the Atherosclerosis Risk in Communities (ARIC) study cohort on the basis of B-mode carotid artery ultrasonograms obtained from 1986 through 1989. Cases exceeded the 90th percentile of IMT, and control subjects were below the 75th percentile of IMT for all arterial segments. Biochemical analyses were performed on fasting frozen (-70 degrees C) serum specimens collected from 1990 through 1992. In conditional logistic regression adjusting for age, blood storage time, total cholesterol, and log-triglyceride concentrations, serum beta-cryptoxanthin and lutein plus zeaxanthin levels were inversely related to the extent of atherosclerosis (odds ratio [OR] per 1-SD increase: 0.75, 95% confidence interval [CI]: 0.59-0.94; and OR per 1-SD increase: 0.76, 95% CI: 0.59-0.95, respectively). Increases in alpha-carotene and lycopene were associated with nonsignificantly lower odds of being a case, whereas beta-carotene, retinol, and alpha-tocopherol were unrelated to IMT. Although not reaching statistical significance, the lag phase and autoantibodies against MDA-LDL were positively associated with asymptomatic atherosclerosis. After adjustment for potential confounders, only the inverse association of lutein plus zeaxanthin with asymptomatic atherosclerosis was maintained. This study supports a modest inverse association between circulating levels of some carotenoids, particularly lutein plus zeaxanthin, and carotid IMT. These findings suggest that these carotenoid compounds (regarded as biomarkers of fruit and vegetable intake) may be important in early stages of atherosclerosis.

#### **Acetylsalicylic acid and vitamin E in prevention of arterial thrombosis.**

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Can J Cardiol (Canada) May 1997, 13 (5) p533-5

Both acetylsalicylic acid and vitamin E have been shown to be beneficial in the prevention of stroke and heart attacks. It is implied that their combination in the treatment of thrombotic complications of atherosclerosis may have added benefits. It is suggested that vitamin E may work as a platelet lysosome stabilizing agent.

#### **Alpha-Tocopherol and beta-carotene serum levels in post-menopausal women treated with transdermal estradiol and oral medroxyprogesterone acetate.**

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Laboratory of Biochemistry, IRCCS S. De Bellis, Castellana G., Italy.  
Horm Metab Res (Germany) Oct 1996, 28 (10) p558-61

Estrogens exert a protective effect against atherosclerosis. It is well known that hormone replacement therapy (HRT) can effectively decrease LDL-cholesterol

and increase HDL-cholesterol and Apo-AI serum levels. Some recent studies have suggested that estrogens alone or in association with progestins may exert an antioxidant effect on lipids. Besides sex steroids, also vitamins exert an antioxidant effect on LDL and may preserve the endogenous antioxidants of LDL. The aim of our study was to evaluate whether HRT can improve alpha-tocopherol and beta-carotene serum levels in post-menopausal women. Fifteen postmenopausal women with climacteric symptoms were treated with 50 micrograms/24 h estradiol transdermally applied twice a week for 21 days. A daily dose of 10 mg oral medroxyprogesterone acetate was added for 12 days in each treatment cycle. This therapy lasted 6 months. A significant reduction was found in total cholesterol and LDL-cholesterol after treatment. Besides, our study has shown that alpha-toc/LDL and beta-car/LDL ratios significantly increased after treatment, while alpha-tocopherol and beta-carotene serum levels did not change significantly after therapy. These preliminary findings suggest that HRT can preserve the content of alpha-tocopherol and beta-carotene in LDL particles and keep the LDL in a reduced antioxidant state.

### **Vitamin E consumption and the risk of coronary disease in women**

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Channing Laboratory, Boston, MA 02115.  
New Engl. J. Med. (USA), 1993, 328/20 (1444-1449)

Background. Interest in the documented 552 cases of major coronary disease (437 nonfatal myocardial infarctions and 115 deaths due to coronary disease).

Results. As compared with women in the lowest fifth of the cohort with respect to vitamin E intake, those in the top fifth had a relative risk of major coronary disease of 0.66 (95 percent confidence interval, 0.50 to 0.87) after adjustment for age and smoking. Further adjustment for a variety of other coronary risk factors and nutrients, including other antioxidants, had little effect on the results. Most of the variability in intake and reduction in risk was attributable to vitamin E consumed as supplements. Women who took vitamin E supplements for short periods had little apparent benefit, but those who took them for more than two years had a relative risk of major coronary disease of 0.59 (95 percent confidence interval, 0.38 to 0.91) after adjustment for age, smoking status, risk factors for coronary disease, and use of other antioxidant nutrients (including multivitamins).

Conclusions. Although these prospective data do not prove a cause-and-effect relation, they suggest that among middle-aged women the use of vitamin E supplements is associated with a reduced risk of coronary heart disease. Randomized trials of vitamin E in the primary and secondary prevention of coronary disease are being conducted; public policy recommendations about the widespread use of vitamin E should await the results of these trials.

## **The role of free radicals in disease**

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Aust N Z J Ophthalmol 1995 Feb;23(1):3-7

Evidence is accumulating that most of the degenerative diseases that afflict humanity have their origin in deleterious free radical reactions. These diseases include atherosclerosis, cancer, inflammatory joint disease, asthma, diabetes, senile dementia and degenerative eye disease. The process of biological ageing might also have a free radical basis. Most free radical damage to cells involves oxygen free radicals or, more generally, activated oxygen species (AOS) which include non-radical species such as singlet oxygen and hydrogen peroxide as well as free radicals. The AOS can damage genetic material, cause lipid peroxidation in cell membranes, and inactivate membrane-bound enzymes. Humans are well endowed with antioxidant defences against AOS; these antioxidants, or free radical scavengers, include ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), beta-carotene, coenzyme Q10, enzymes such as catalase and superoxide dismutase, and trace elements including selenium and zinc. The eye is an organ with intense AOS activity, and it requires high levels of antioxidants to protect its unsaturated fatty acids. The human species is not genetically adapted to survive past middle age, and it appears that antioxidant supplementation of our diet is needed to ensure a more healthy elderly population.

## **Coenzyme Q10 and coronary artery disease**

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Clin Investig 1993;71(8 Suppl):S112-5

It has been postulated that oxidatively modified low-density lipoprotein (LDL) contributes to the genesis of atherosclerosis. Ubiquinone has been suggested to be an important physiological lipid-soluble antioxidant and is found in LDL fractions in the blood. We measured plasma level of ubiquinone using high-performance liquid chromatography and plasma levels of total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides in 245 normal subjects (186 males, 59 females) and in 104 patients (55 males, 49 females) who had coronary artery disease not receiving pravastatin and 29 patients (12 males, 17 females) receiving pravastatin. In the normal subjects, the plasma ubiquinone levels did not vary with age. In the patient groups, the plasma total cholesterol and LDL levels were higher and the plasma ubiquinone level lower than in the normal subject group. The LDL/ubiquinone ratio was higher in the patient groups. We found that ubiquinone level, either alone or when expressed in relation to LDL levels, was significantly lower in the patient groups compared with the normal subject group. The 3-hydroxy-3-methylglutaryl coenzyme A (HMC CoA) reductase inhibitor is

thought to prevent atherosclerosis, however, it also inhibits ubiquinone production. The present study revealed that HMG CoA reductase inhibitor decreased plasma cholesterol level, and that it did not improve either the ubiquinone level or the LDL/ubiquinone ratio. From these results, the LDL/ubiquinone ratio is likely to be a risk factor for atherogenesis, and administration of ubiquinone to patients at risk might be needed.

- Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women

New England Journal of Medicine (USA), 1996, 334/18

**Background.** The role of dietary antioxidant vitamins in preventing coronary heart disease has aroused considerable interest because of the knowledge that oxidative modification of low-density lipoprotein may promote atherosclerosis. **Methods.** We studied 34,486 postmenopausal women with no cardiovascular disease who in early 1986 completed a questionnaire that assessed, among other factors, their intake of vitamins A, E, and C from food sources and supplements. During approximately seven years of follow-up (ending December 31, 1992), 242 of the women died of coronary heart disease. **Results.** In analyses adjusted for age and dietary energy intake, vitamin E consumption appeared to be inversely associated with the risk of death from coronary heart disease. This association was particularly striking in the subgroup of 21,809 women who did not consume vitamin supplements (relative risks from lowest to highest quintile of vitamin E intake, 1.0, 0.68, 0.71, 0.42, and 0.42; P for trend = 0.008). After adjustment for possible confounding variables, this inverse association remained (relative risks from lowest to highest quintile, 1.0, 0.70, 0.76, 0.32, and 0.38; P for trend = 0.004). There was little evidence that the intake of vitamin E from supplements was associated with a decreased risk of death from coronary heart disease, but the effects of high-dose supplementation and the duration of supplement use could not be definitively addressed. Intake of vitamins A and C did not appear to be associated with the risk of death from coronary heart disease. **Conclusions.** These results suggest that in postmenopausal women the intake of vitamin E from food is inversely associated with the risk of death from coronary heart disease and that such women can lower their risk without using vitamin supplements. By contrast, the intake of vitamins A and C was not associated with lower risks of dying from coronary disease.

**Randomized, controlled trial of antioxidant vitamins and cardioprotective diet on hyperlipidemia, oxidative stress, and development of experimental atherosclerosis: The diet and antioxidant trial on atherosclerosis (DATA)**

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Cardiovasc Drugs Ther 1995 Dec;9(6):763-71

The effects of administration of guava and papaya fruit (100 g/day), vegetables, and mustard oil (5 g/day) (group A); antioxidant vitamins C (50 mg/day) and E (30 mg/day) plus betacarotene (10 mg/day) (group B); a high-fat (5-10 g/day) (group C); or a low-fat (4-5 g/day) diet (group D) were compared over 24 diet weeks in a randomized fashion, while all groups of rabbits (five in each of four groups) received a hydrogenated fat diet (5-10 g/day) for a period of 36 weeks. After 12 weeks on the high fat diet, each group of rabbits had an increase in blood lipoproteins. The fruit and vegetable-enriched prudent diet (group A) caused a significant decline in blood lipids at 24 and 36 weeks, whereas the lipid levels increased significantly in groups C and D. Group A also had a significant rise in vitamin E (2.1 Umol/l), C (10.5 Umol/l), A (0.66 Umol/l), and carotene (0.08 Umol/l) and a decrease in lipid peroxides (0.34 nmol/ml at 36 weeks, whereas the levels were unchanged in groups C and D. Group B rabbits had a significant and greater increase than group A in plasma vitamins E, C, A, and carotene; a rise in HDL cholesterol; and a greater decrease in lipid peroxides after 24 and 36 weeks of treatment. After stimulation of lipid peroxidation in all rabbits, 3 of 5 group C and 2 of 5 group D rabbits died due to coronary thrombosis, whereas in groups A and B there were no deaths, indicating that antioxidant therapy can provide protection against lipid peroxidation and free radical generation. Aortic lipids and sudanophilia, indicating atherosclerosis, were significantly higher in groups C and D than in groups A and B. Fatty streaks and atheromatous and fibrous plaques were noted in all the rabbits in groups C and D. Intimal fibrosis and medial degeneration were also present in the group C rabbits. While group A (36.4 plus or minus 4.4 microm) and group B (37.1 plus or minus 4.2 microm) rabbits had minimal coronary artery plaque sizes, group C (75.4 plus or minus 10.6 microm) and group D rabbits (69.5 plus or minus 6.2 microm) had significantly greater plaque sizes. Aortic plaque sizes were also greater in groups C and D than in groups A and B. It is possible that combined therapy with antioxidant vitamins C, E, and carotene, and a diet rich in antioxidants, could independently inhibit free radical generation and the development of atherosclerosis.

### **Serum levels of vitamin E in relation to cardiovascular diseases**

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J Clin Pharm Ther 1995 Dec;20(6):335-40

Serum vitamin E levels in healthy people (n = 71) and patients with cardiovascular diseases (n = 62) were determined. The cases of cardiovascular disease comprised patients with acute myocardial infarction (AMI) (n = 31), atherosclerosis (AT) (n = 23) and myocardial ischaemia (MI) (n = 8). The mean (plus or minus SD) serum vitamin E levels of the control group and the group with cardiovascular disease were 1.12 plus or minus 0.27 mg% and 0.98 plus or minus 0.41 mg%, respectively. Patients with AMI, AT and MI had corresponding levels of 0.97 plus or minus 0.48 mg%, 1.00 plus or minus 0.39 mg% and 1.01 plus or minus 0.44 mg%, respectively. Overall serum vitamin E levels were lower in the group with cardiovascular disease than in the control group. Patients and the

control group are also discussed with respect to a number of potentially confounding parameters such as age, sex, smoking status, quetelet index (kg/m<sup>2</sup>), alcohol consumption, dietary intake and serum lipids.

### **Oxidative susceptibility of low density lipoprotein from rabbits fed atherogenic diets containing coconut, palm, or soybean oils**

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*Lipids* (USA), 1995, 30/12 (1145-1150)

The oxidative susceptibilities of low density lipoproteins (LDL) isolated from rabbits fed high-fat atherogenic diets containing coconut, palm, or soybean oils were investigated. New Zealand white rabbits were fed atherogenic semisynthetic diets containing 0.5% cholesterol and either (i) 13% coconut oil and 2% corn oil (CNO), (ii) 15% refined, bleached, and deodorized palm olein (RBDPO), (iii) 15% crude palm olein (CPO), (iv) 15% soybean oil (SO), or (v) 15% refined, bleached, and deodorized palm olein without cholesterol supplementation (RBDPO(wc)), for a period of twelve weeks. Total fatty acid compositions of the plasma and LDL were found to be modulated (but not too drastically) by the nature of the dietary fats. Cholesterol supplementation significantly increased the plasma level of vitamin E and effectively altered the plasma composition of long-chain fatty acids in favor of increasing oleic acid. Oxidative susceptibilities of LDL samples were determined by Cu<sup>2+</sup>-catalyzed oxidation which provide the lag times and lag-phase slopes. The plasma LDL from all palm oil diets (RBDPO, CPO, and RBDPO(wc)) were shown to be equally resistant to the oxidation, and the LDL from SO-fed rabbits were most susceptible, followed by the LDL from the CNO-fed rabbits. These results reflect a relationship between the oxidative susceptibility of LDL due to a combination of the levels of polyunsaturated fatty acids and vitamin E.

### **Coantioxidants make alpha-tocopherol an efficient antioxidant for low-density lipoprotein**

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*Am J Clin Nutr* 1995 Dec;62(6 Suppl):1357S-1364S

The oxidation of low-density lipoproteins (LDLs) is now commonly implicated as an important early event in atherogenesis. The resulting interest in LDL antioxidation has focused on alpha-tocopherol, the biologically and chemically most active form of vitamin E and quantitatively the major lipid-soluble antioxidant in extracts prepared from human LDL. We review advances made in our understanding of the molecular action of alpha-tocopherol in radical-mediated

oxidation of isolated human LDL and how the vitamin's antioxidant activity is enhanced or even dependent on the presence of suitable reducing species, which are referred to as coantioxidants.

### **Optimal diet for reducing the risk of arteriosclerosis**

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Can J Cardiol 1995 Oct;11 Suppl G:118G-122G

The primary objectives of current dietary advice for those at risk from coronary artery disease (CAD) focus on progressive restriction of dietary saturated (and trans) fatty acids and cholesterol intake, combined with exercise and achievement of ideal body weight. These principles are endorsed by the official bodies of most western nations concerned with reducing CAD mortality and have recently been reaffirmed by the Adult Treatment Panel of the National Cholesterol Education Program. There has been concern, however, in view of the increasing use of drug therapy, that additional strategies should supplement the primary goals to increase the palatability and effectiveness of the diet. These additional strategies include increased intake of foods high in soluble viscous fibres, vegetable proteins, possibly antioxidants such as vitamin E and the isoflavonoids, increased intake of alpha-linolenic acid and, for those with low high density lipoprotein cholesterol levels, increased monounsaturated fat intake. These strategies translate into advice to significantly increase consumption of specific plant foods such as green leafy vegetables, nuts and seeds, and dried legumes, all of which improve the overall nutritional quality of the diet and contain specific active ingredients. These changes represent a regression to a more primitive diet on the evolutionary scale.

### **Effect of vitamin E, vitamin C and beta-carotene on LDL oxidation and atherosclerosis**

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Canadian Journal of Cardiology (Canada), 1995, 11/Suppl. G (97G-103G)

**OBJECTIVE:** The oxidative modification of low density lipoprotein (LDL) may be early step in atherogenesis. Furthermore, evidence of oxidized LDL has been found in vivo. The most persuasive evidence shows that supplementation of some animal models with antioxidants slows atherosclerosis. The purpose of this review is to examine the roles that vitamin E, vitamin C and beta-carotene may play in reducing LDL oxidation.

**DATA SOURCES:** English language articles published since 1980, particularly from groups active in this field of research.

**STUDY SELECTION:** In vitro, animal, and human studies on antioxidants, LDL oxidation, and atherosclerosis were selected.

**DATA SYNTHESIS:** Vitamin E has shown the most consistent effects with regard to LDL oxidation. Beta-carotene appears to have only a mild or no effect on oxidizability. Ascorbate, although it is not lipophilic, can also reduce LDL oxidative susceptibility.

**CONCLUSIONS:** LDL oxidizability can be reduced by antioxidant nutrients. However, more research is needed to establish their utility in the prevention of coronary artery disease.

### **Atherosclerosis: Vitamin E protects coronary arteries**

Trapp R., Germany  
Deutsche Apotheker Zeitung (Germany), 1995, 135/41 (42+44)

No abstract.

### **Effects on health of dietary supplementation with 100 mg d-alpha-tocopheryl acetate, daily for 6 years**

Takamatsu S; Takamatsu M; Satoh K; Imaizumi T; Yoshida H; Hiramoto M; Koyama M; Ohgushi Y; Mizuno S  
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J Int Med Res 1995 Sep-Oct;23(5):342-57

To evaluate the clinical antioxidant effects of vitamin E, 161 healthy volunteers aged 39 to 56 years, were given 100 or 3 mg of d-alpha-tocopheryl acetate orally daily for 6 years using a randomized, double-blind design, among the 147 volunteers who qualified for the analysis, seven of the 73 volunteers receiving 3 mg d-alpha-tocopheryl acetate daily and none of the 74 volunteers receiving 100 mg had coronary disorders including myocardial damage ( $P < 0.02$ ). ST or T wave abnormalities on electrocardiograms were considered to indicate coronary disorders (four volunteers). The mean serum total tocopherol (TOC) concentration in the 100-mg group was significantly higher than that in the 3-mg group 6 months after the start of the study, and this raised value was maintained throughout the study; the level in the 3-mg group did not change significantly from the baseline value. The low-density lipoprotein cholesterol/total TOC ratio, a parameter of the inhibition of peroxidation of low-density lipoprotein cholesterol, was the only serum lipid parameter that was significantly different, at baseline, in

the volunteers with coronary disorders compared with the others. These findings indicate that long-term supplementation with 100 mg tocopheryl acetate daily may prevent the early stages of coronary atherosclerosis by decreasing peroxidation of low-density lipoprotein cholesterol.

### **Mechanisms of the cardioprotective effect of a diet enriched with omega-3 polyunsaturated fatty acids**

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Pathophysiology (Netherlands) 1995, 2/3 (131-140)

The review presents current views of metabolic conversions of class omega-3 polyunsaturated fatty acids (omega-3 PUFA) and their effects on the heart function. The role of these compounds in regulation of the membrane lipid composition is discussed. Within the organism, omega-3 PUFA incorporate more effectively into membrane phospholipids of the myocardium in comparison with other organs. In animals kept on a omega-3 PUFA-enriched diet, the intramembrane concentration of omega-6 PUFA, in the first place, of rachidonic acid, decreases. Substitution of omega-3 PUFA for arachidonic acid in the metabolic system of eicosanoid synthesis initiates the synthesis of prostaglandins and thromboxanes possessing lowered biological activity, thus minimizing the risk of clot formation in the cardiovascular system. As omega-3 PUFA are direct substrates for lipid peroxidation, any rise in omega-3 PUFA concentration sharply activates free-radical oxidation in the membranes of internal organs particularly in the liver. Original data are presented that in rats kept on omega-3 PUFA-enriched diets, the kinetic parameters of the Ca<sup>2+</sup> transport system do not change. However, the resistance of the system to free radical oxidation increases considerably. This may increase myocardial resistance to free-radical-dependent injuries. A rise in the intramembrane omega-3 PUFA content which brings about structural rearrangements within lipids and changes the activity of membrane-bound enzymes in vitro, has no effect in vivo. This finding points to the existence of a mechanism compensating for changes in the fatty acid composition of foods. Data from literature analysis suggest that one of the most active participants in the compensatory system is alpha-tocopherol, a lipid peroxidation inhibitor and a structural stabilizer of biomembranes. With a rise in omega-3 PUFA concentration, alpha-tocopherol is released from the liver and blood flow and accumulated in the body (predominantly in myocardial membranes). Whereas potent chemical antioxidants display an ability to inhibit physiologically important free-radical reactions occurring in the organism, vitamin E is without side effects even when used at high concentrations. In case of long-term application of omega-3 PUFA-enriched diets, alpha-tocopherol must be added to the diet.

### **Prevention of atherosclerosis: The potential role of antioxidants**

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Postgrad Med 1995 Jul;98(1):175-6, 179-84

Evidence is increasing that oxidation of low-density lipoprotein cholesterol may be instrumental in atherogenesis. As a result a number of studies have been undertaken to evaluate the effects of antioxidant vitamins, beta carotene, selenium, and monounsaturated fat on coronary artery disease. Results in many instances have been promising, particularly in the case of vitamin E supplements. Studies of pro-oxidants, such as iron and copper, are inconclusive at this time, and a trial to assess the value of probucol in hypercholesterolemic patients is currently under way.

### **Vitamin E: Metabolism and role in atherosclerosis**

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Ann Biol Clin (Paris) 1994;52(7-8):515-22

Vitamin E is the term used for eight naturally occurring fat-soluble nutrients. Alpha-tocopherol predominates in many species and has the highest biological activity. Vitamin E is absorbed via the lymphatic pathway and transported in association with CM. Vitamin E is carried in plasma by lipoproteins. It is secreted by the liver in nascent VLDL with a preferential incorporation of alpha-tocopherol. Most of the plasma vitamin E is in LDL and in HDL. Vitamin E is exchanged readily between lipoproteins: tocopherol in HDL readily transfers to apolipoprotein B-containing lipoproteins (VLDL, LDL), with little return of tocopherol from the apolipoprotein B-containing lipoproteins to HDL. The mechanisms of tissue uptake of vitamin E from the lipoproteins is poorly understood. This uptake may occur during catabolism of triacylglycerol-rich lipoproteins by the activity of lipoprotein lipase, via the LDL receptor or by nonreceptor-mediated uptake. Vitamin E may act to prevent the initiation/progression of spontaneous atherosclerosis. This concept is based on in-vitro data: vitamin E influences the responses of cells (vascular endothelial cells, leukocytes, vascular smooth muscle cells) and the modification of lipoproteins (especially LDL) which, at least in principle, could contribute to the initiation/progression of spontaneous atherosclerosis. In vivo studies are clearly required to establish the extent and mode of vitamin E's antiatherosclerotic impact and, hence, its therapeutic potential.

## **Vitamin C prevents cigarette smoke-induced leukocyte aggregation and adhesion to endothelium in vivo**

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Proc. Natl. Acad. Sci. U.S.A. (USA), 1994, 91/16 (7688-7692)

A common feature of cigarette-smoke (CS)-associated diseases such as atherosclerosis and pulmonary emphysema is the activation, aggregation, and adhesion of leukocytes to micro- and macrovascular endothelium. A previous study, using a skinfold chamber model for intravital fluorescence microscopy in awake hamsters, has shown that exposure of hamsters to the smoke generated by one research cigarette elicits the adhesion of fluorescently labeled leukocytes to the endothelium of arterioles and small venules. By the combined use of intravital microscopy and scanning electron microscopy, we now demonstrate in the same animal model that (i) CS-induced leukocyte adhesion is not confined to the microcirculation, but that leukocytes also adhere singly and in clusters to the aortic endothelium; (ii) CS induces the formation in the bloodstream of aggregates between leukocytes and platelets; and (iii) CS-induced leukocyte adhesion to micro- and macrovascular endothelium and leukocyte-platelet aggregate formation are almost entirely prevented by dietary or intravenous pretreatment with the water-soluble antioxidant vitamin C (venules, 21.4 plus or minus 11.0 vs. 149.6 plus or minus 38.7 leukocytes per mm<sup>2</sup>,  $P < 0.01$ ; arterioles, 8.5 plus or minus 4.2 vs. 54.3 plus or minus 21.6 leukocytes per mm<sup>2</sup>,  $P < 0.01$ ; aortas, 0.8 plus or minus 0.4 vs. 12.4 plus or minus 5.6 leukocytes per mm<sup>2</sup>,  $P < 0.01$ ; means plus or minus SD of  $n = 7$  animals, 15 min after CS exposure). No inhibitory effect was observed by pretreatment of the animals with the lipid-soluble antioxidants vitamin E or probucol. The protective effects of vitamin C on CS-induced leukocyte adhesion and aggregation were seen at vitamin C plasma levels (55.6 plus or minus 22.2 microM,  $n = 7$ ) that can easily be reached in humans by dietary means or supplementation, suggesting that vitamin C effectively contributes to protection from CS-associated cardiovascular and pulmonary diseases in humans.

## **Hyperhomocysteinaemia: a role in the accelerated atherogenesis of chronic renal failure?**

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Neth J Med (Netherlands) May 1995, 46 (5) p244-51

Moderate hyperhomocysteinaemia has recently been established as an independent risk factor for atherothrombotic disease. It might be caused by heterozygosity for cystathionine beta-synthase deficiency, an enzyme involved in the conversion of methionine to cysteine through the transsulphuration pathway or by inherited thermolability of the enzyme which remethylates homocysteine into

methionine. In chronic renal failure (CRF) homocysteine levels are significantly elevated at a relatively early stage. The normal kidney possibly plays an important role in homocysteine catabolism, which cannot be performed in CRF. Alternatively, decreased extrarenal catabolism can contribute to the hyperhomocysteinaemia in this disease state. Treatment with folic acid, 5 mg daily, significantly lowers homocysteine levels in chronic renal patients. (45 Refs.)

### **Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease.**

Van den Berg M, Boers GH, Franken DG, Blom HJ, Van Kamp GJ, Jakobs C, Rauwerda JA, Kluft C, Stehouwert CD  
Department of Vascular Surgery, Free University Hospital, Amsterdam, The Netherlands.  
Eur J Clin Invest (England) Mar 1995, 25 (3) p176-81

Hyperhomocysteinaemia, defined as an abnormally high plasma homocysteine concentration after an oral methionine load, is common in young (< or = 50 years) patients with peripheral arterial occlusive disease. It is thought to predispose to atherosclerosis by injuring the vascular endothelium. Treatment with pyridoxine and/or folic acid may lower plasma homocysteine levels. In mildly hyperhomocysteinaemic patients with peripheral arterial occlusive disease, we studied the effect of daily treatment with pyridoxine (250 mg) plus folic acid (5 mg) on homocysteine metabolism (i.e. plasma concentrations in the fasting state and after methionine loading, in 48 patients) and on endothelial function (in 18 patients). Endothelial function was estimated as the plasma concentrations of the endothelium-derived proteins, von Willebrand factor (vWF), thrombomodulin ?, and tissue-type plasminogen activator (tPA). At baseline, fasting homocysteine levels were above normal in 24 of the 48 patients (50%); post-load levels, by definition, were above normal in 100% of patients. After 12 weeks of treatment, fasting and post-load levels were normal in 98 and 100% of patients, respectively. Endothelial function was assessed in 18 patients who completed 1 year of treatment. At baseline, median vWF (235%) and TM (57.1 ng mL<sup>-1</sup>) levels were above normal. At follow-up, vWF levels had decreased to 170% (P = 0.01) and TM levels had decreased to 49 ng mL<sup>-1</sup> (P = 0.04). tPA levels were normal at baseline and did not change. Endothelial dysfunction is present in young patients with peripheral arterial occlusive disease and hyperhomocysteinaemia. Pyridoxine plus folic acid treatment normalizes homocysteine metabolism in virtually all patients, and appears to ameliorate endothelial dysfunction.

### **Vitamin nutrition status and homocysteine: an atherogenic risk factor.**

Ubbink JB

Department of Chemical Pathology, University of Pretoria, South Africa.  
Nutr Rev (United States) Nov 1994, 52 (11) p383-7

In an epidemiologic survey, a marginal status of folic acid, vitamin B12, and vitamin B6 was shown to be associated with hyperhomocysteinemia. In a case-control study, a low plasma folate concentration was associated with increased coronary heart disease risk. This phenomenon appears to be mediated by folate's effect on homocysteine metabolism. Both studies offer further perspectives on homocysteine as an atherogenic risk factor.

### **Homocysteine and coronary artery disease.**

Robinson K; Mayer E; Jacobsen DW

Department of Cardiology, Cleveland Clinic Foundation, OH 44195.  
Cleve Clin J Med (United States) Nov-Dec 1994, 61 (6) p438-50

**BACKGROUND** Homocystinuria is a rare autosomal recessive disease complicated by early and aggressive occlusive arterial disease. This may be related to the grossly increased homocysteine concentrations seen in this disease. More recently, milder hyperhomocysteinemia has been proposed as an independent risk factor for coronary artery disease.

**SUMMARY:** Many patients with homozygous homocystinuria develop severe premature atherosclerosis and thromboembolism, probably caused by abnormally high concentrations of homocysteine. Homocysteine undergoes metabolism either by remethylation or transsulfuration, and deficiency or dysfunction of any of the substances that regulate these reactions may lead to hyperhomocysteinemia. Homocysteine may have adverse effects on platelets, clotting factors, and endothelial cells. Studies have demonstrated significantly higher plasma homocysteine levels in patients with occlusive arterial disease than in controls. The causes are not clearly understood but may include deficiency of vitamin B6, vitamin B12, and folic acid and heterozygosity for cystathionine synthase deficiency. Vitamin supplementation can lower plasma homocysteine levels.

**CONCLUSIONS:** Whether measuring plasma homocysteine levels in patients with coronary artery disease should be routine and whether treating hyperhomocysteinemia in these patients may reduce the risk of coronary events remains to be determined. (85 Refs.)

### **Platelets, carotids, and coronaries. Critique on antithrombotic role of antiplatelet agents, exercise, and certain diets.**

Eichner ER

Am J Med (United States) Sep 1984, 77 (3) p513-23

"Antiplatelet" drugs and certain life styles seem to have an "antithrombotic" effect that may help protect against stroke and heart attack. This review of the experience with aspirin, dipyridamole, and sulfinpyrazone offers new interpretations of some of the major clinical trials, suggests guidelines for use of antiplatelet drugs, and integrates novel observations on diet and exercise into the "thromboxane- prostacyclin balance" hypothesis. It is argued that the Canadian stroke study showed that aspirin protects men with transient ischemic attacks from coronary death as well as from stroke, that type II errors may have been made in some clinical trials, that aspirin protects women as well as men, that aspirin benefits patients who have had a heart attack, that the effect of aspirin in angina varies with the type of angina, that the dose of aspirin used may not be critical, that guidelines for use of dipyridamole and sulfinpyrazone are still inconclusive, and that exercise and fish oil supplements may be "antithrombotic." (100 Refs.)

### **Effects of 11-week increases in dietary eicosapentaenoic acid on bleeding time, lipids, and platelet aggregation.**

Thorngren M; Gustafson A  
Lancet (England) Nov 28 1981, 2 (8257) p1190-3

The effect of a diet rich in eicosapentaenoic acid (EPA) on platelet phospholipid fatty acid composition, platelet aggregation, and bleeding time was studied in 10 healthy men, whose usual diet was partly replaced by fish for 11 weeks. This diet provided 2-3 g EPA per day. Two doses (3.5 and 10 mg/kg body-weight) of acetylsalicylic acid (ASA) were given before and during the diet. The fish diet prolonged bleeding time (by 42%) and decreased platelet aggregability. The changes in platelet phospholipid fatty acid composition consisted of increases in the omega-3 series (C20:5 and C22:6) and decreases in the omega-6 series (C18:2 and C20:3). The reduction in platelet aggregation induced by collagen and ADP did not parallel the changes in platelet membrane phospholipids and bleeding times. Diminished platelet aggregation induced by collagen lasted only 3 weeks (while subject was still on the diet), whereas the decreased sensitivity to ADP persisted for at least 11 weeks after the volunteers had resumed their normal diet. ASA taken before the diet prolonged bleeding time by as much as did the diet itself. ASA taken during the diet prolonged bleeding time by more than the sum of the increases in bleeding time caused by ASA and by the EPA diet separately, but the synergism was not significantly more than additive. The findings suggest that a diet rich in omega-3 polyunsaturated fatty acids reduces the interaction between platelets and the vessel wall by mechanisms which are more complex than just a reduction in susceptibility of platelets to the naturally occurring agents collagen and ADP, or an imbalance between proaggregatory and anti-aggregatory prostaglandin derivatives.

**N-3 but not N-6 fatty acids reduce the expression of the combined adhesion and scavenger receptor CD36 in human monocytic cells.**

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Cell Biochem Funct (England) Sep 1995, 13 (3) p211-6

CD36, a multifunctional adhesion receptor e.g. for thrombospondin and collagen, as well as a scavenger receptor for oxidized low density lipoprotein, is expressed e.g. on platelets and monocytes. By this dual role it might be involved in early steps of atherosclerosis like the recruitment of monocytes and formation of foam cells. We therefore studied the effects of n-3 fatty acids on CD36 expression in human monocytic cells. Incorporation of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) into cellular phospholipids resulted in a significant reduction of CD36 expression at the mRNA and protein level, whereas arachidonic acid (AA, C20: 4n-6) and linoleic acid (LA, C18:2n-6) tended to increase CD36 expression compared to the control. This specific down-regulation of CD36 by n-3 fatty acids in cells involved in the initiation and progression of atherogenesis and inflammation, represents a further mechanism that may contribute to the beneficial effects of n- 3 polyunsaturated fatty acids (PUFA) in these disorders.

**Essential fatty acid metabolism in patients with essential hypertension, diabetes mellitus and coronary heart disease.**

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Prostaglandins Leukot Essent Fatty Acids (Scotland) Jun 1995, 52 (6) p387-91

Mortality and morbidity from coronary heart disease (CHD), diabetes mellitus (DM) and essential hypertension (HTN) are higher in people of South Asian descent than in other groups. There is evidence to believe that essential fatty acids (EFAs) and their metabolites may have a role in the pathobiology of CHD, DM and HTN. Fatty acid analysis of the plasma phospholipid fraction revealed that in CHD the levels of gamma- linolenic acid (GLA), arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are low, in patients with HTN linoleic acid (LA) and AA are low, and in patients with non-insulin dependent diabetes mellitus (NIDDM) and diabetic nephropathy the levels of dihomo-gamma-linolenic acid (DGLA), AA, alpha-linolenic acid (ALA) and DHA are low, all compared to normal controls. These results are interesting since DGLA, AA and EPA form precursors to prostaglandin E1, (PGE1), prostacyclin (PGI2), and PGI3, which are potent platelet anti- aggregators and vasodilators and can prevent thrombosis and atherosclerosis. Further, the levels of lipid peroxides were found to be high in patients with CHD, HTN, NIDDM and diabetic nephropathy. These results suggest that increased formation of lipid peroxides and

an alteration in the metabolism of EFAs are closely associated with CHD, HTN and NIDDM in Indians.

**[Changes in fatty acid composition, platelet aggregability and RBC function in elderly subjects with administration of low-dose fish oil concentrate and comparison with younger subjects]**

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Nippon  
Ronen Igakkai Zasshi (Japan) Aug 1994, 31 (8) p596-603

Anti-thrombotic and anti-atherogenic effects of eicosapentaenoic acid (EPA) through the modulation of various cell functions related to thrombogenesis have been reported recently. We previously reported that the administration of EPA at low doses could more effectively elevate the plasma EPA concentration in elderly subjects than in younger ones. Magnetic resonance imaging examination of the brain often reveals lacunar lesions in elderly subjects without any signs or symptoms of cerebrovascular diseases. In this study we clarified the effect of administration of low doses of fish oil concentrate on platelet and RBC function in elderly subjects, compared with younger subjects. Thirty six elderly subjects (mean age 78) without any signs or symptoms of cerebrovascular diseases, all receiving the same diet in the same lodging house for the aged, were divided into 3 groups. Different amounts of fish oil concentrate (0.25-0.5 g/day of EPA) were administered to the 3 groups, daily for more than 1 month. Changes of plasma fatty acid composition, platelet aggregability, whole blood viscosity and RBC deformability was examined before and after EPA administration. One month after EPA treatment, the plasma EPA content had increased dose dependently, with suppression of platelet aggregation and improvement of RBC function. In younger subjects receiving the same amount of EPA, the elevation of plasma EPA was less than that observed in the elderly. In summary, low dose EPA administration can improve the function of platelet and RBC to an anti-thrombotic state and would be useful to prevent the occurrence of cerebrovascular diseases in elderly subjects without any side effects.

**Do fish oils prevent restenosis after coronary angioplasty?**

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Circulation (United States) Nov 1994, 90 (5) p2248-57

BACKGROUND-The omega-3 polyunsaturated fatty acids derived from fish oils have been shown to modulate many factors believed to affect the pathogenesis of atherosclerosis. Because certain features of restenosis following angioplasty

mimic some of the early changes of atherogenesis, some researchers have suggested that fish oil might prevent restenosis following angioplasty. We report the effects of omega-3 fatty acids on the rate of restenosis following percutaneous intraluminal coronary angioplasty (PTCA).

**METHODS AND RESULTS**-From August 1989 through September 1992, 551 patients were randomized to start receiving a daily dietary supplement of ten 1.0-g capsules containing 80.6% ethyl esters of omega-3 fatty acids providing 4.1 g eicosapentaenoic acid (EPA) and 2.8 g docosahexaenoic acid (DHA) for 6 months or an equal amount of an ethyl ester of corn oil. Four hundred seventy subjects who were well matched for risk factors completed successful angioplasty of one or multiple lesions in native coronary vessels and constituted the study cohort, of whom 447 were evaluable at 6 months after PTCA. The criteria for restenosis were that the quantitative coronary angiography at 6 months show a > 30% increase in narrowing at the stenosis site or loss of at least half of the gain achieved at the time of PTCA and final restenosis with < 50% luminal diameter remaining. In 93% of the patients, the end point was determined by angiography and in all except 1% of these by quantitative coronary angiography. Compliance with the fish oil supplement was good as judged by incorporation of EPA and DHA in plasma and red blood cell phospholipids. The restenosis rate among analyzable patients was 46% for corn oil and 52% for fish oil ( $P = .37$ ). The addition of 200 mg alpha-tocopherol for all subjects during the study had no effect on restenosis rates.

**CONCLUSIONS**-This was the largest of such trials to date, and a supplement of 8 g/d of omega-3 fatty acids failed to prevent the usual high rate of restenosis after PTCA. No adverse effects were attributable to this large daily supplement of omega-3 fatty acids.

**n-3 fatty acid incorporation into LDL particles renders them more susceptible to oxidation in vitro but not necessarily more atherogenic in vivo.**

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*Arterioscler Thromb (United States)* Jul 1994, 14 (7) p1170-6

The hypothesis that n-3 fatty acid incorporation into low-density lipoprotein (LDL) particles renders them more susceptible to oxidative modification and possibly more atherogenic was tested using two groups of female Yucatan miniature swine (10 animals per group) fed an atherogenic diet for 8 months. As a supplement to the atherogenic diet, the first group received a daily oral dose of the fish oil (FO) concentrate MaxEPA, rich in n-3 fatty acids, while the second group received the same dosage of a control oil (CO) low in n-3 fatty acids but with the same ratio of polyunsaturated to monounsaturated to saturated fatty acids as MaxEPA. At 8 months, the animals were killed and perfusion fixed, and all major vessels were removed for morphological assessment of atherosclerotic lesion area. Before fixation, blood samples were collected from all 20 pigs, and LDL (d =

1.019 to 1.063 g/mL) was separated from the plasma by ultracentrifugation. A series of in vitro oxidative modification reactions were carried out by incubating the LDL with a copper sulfate solution. The susceptibility of each LDL preparation to oxidation was determined by measuring both the formation of conjugated dienes and the relative mobility of each sample in an agarose gel. The incorporation of n-3 fatty acids into LDL particles decreased the lag phase by 30%, resulting in an increased mobility of FO-LDL (compared with CO-LDL) when incubated for 0.5 to 12 hours, but at longer incubation times (18 to 24 hours), the extent of modification between the two groups became equal.

### **Human atherosclerotic plaque contains both oxidized lipids and relatively large amounts of alpha-tocopherol and ascorbate.**

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Arterioscler Thromb Vasc Biol (United States) Oct 1995, 15 (10) p1616-24

We assessed the antioxidant status and contents of unoxidized and oxidized lipids in freshly obtained, homogenized samples of both normal human iliac arteries and carotid and femoral atherosclerotic plaque. Optimal sample preparation involved homogenization of human atherosclerotic plaque for 5 minutes, which resulted in recovery of most of the unoxidized and oxidized lipids without substantial destruction of endogenous vitamins C and E and 87% and 43% recoveries of added standards of alpha-tocotrienol and isoascorbate, respectively. The total protein, lipid, and antioxidant levels obtained from human plaque varied among donors, although the reproducibility of replicates from a single sample was within 3%, except for ubiquinone-10 and ascorbate, which varied by 20% and 25%, respectively. Plaque samples contained significantly more ascorbate and urate than control arteries, with no discernible difference in the vitamin C redox status between plaque and control materials. The concentrations of alpha-tocopherol and ubiquinone-10 were comparable in plaque samples and control arteries. However, approximately 9 mol percent of plaque alpha-tocopherol was present as alpha-tocopherylquinone, whereas this oxidation product of vitamin E was not detectable in control arteries. Coenzyme Q10 in plaque and control arteries was only detected in the oxidized form ubiquinone-10, although coenzyme Q10 oxidation may have occurred during processing. The most abundant of all studied lipids in plaque samples was free cholesterol, followed by cholesteryl oleate and cholesteryl linoleate (Ch18:2). Approximately 30% of plaque Ch18:2 was oxidized, with 17%, 12%, and 1% present as fatty acyl hydroxides, ketones, and hydroperoxides, respectively.

### **Hyperhomocysteinaemia and end stage renal disease**

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J Nephrol 1997 Mar-Apr;10(2):77-84

Vascular disease is a major cause of morbidity and mortality in end stage renal failure patients and cannot be explained entirely by the prevalence of traditional risk factors for atherosclerosis. A high plasma homocysteine concentration, which is a risk factor for vascular disease is found in patients with end stage renal disease. The exact cause for the hyperhomocysteinaemia seen in these patients is unknown, al metabolism of homocysteine. High homocysteine concentrations may also be attributable to a deficiency of folate, vitamin B6 or vitamin B12 although, because of supplementation, these vitamins may be present in high concentrations in renal patients. The occurrence of hyperhomocysteinaemia despite high plasma vitamin concentration could be due to altered metabolism or inhibition of intracellular vitamin activity. A number of studies have now established hyperhomocystinaemia to be an independent risk factor for atherosclerosis in patients with end-stage renal disease. Plasma homocysteine concentrations can be reduced by administration of folic acid either alone or combined with vitamin B12 or vitamin B6. The effects of such reduction on vascular risk in renal failure patients needs further study.

### **Dietary pectin influences fibrin network structure in hypercholesterolaemic subjects**

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Thrombosis Research (United Kingdom), 1997, 86/3 (183-196)

Fibrinogen is an important risk factor for atherosclerosis, stroke and cardiovascular heart disease (CHD). This risk is increased when associated with a high serum cholesterol. Furthermore, it is also believed that not only fibrinogen concentration, but also the quality of fibrin networks may be an important risk factor for the development of CHD. CHD and stroke as a result of atherosclerosis, plus the related problems of hyperinsulinaemia, hyperlipidaemia and hypertension are strongly related to diet. The 'western' diet, defined by low fibre and high fat, sucrose and animal protein intakes, appears to be a major factor leading to death. It has been established that the water-soluble dietary fibre, pectin, significantly decrease the concentration of serum cholesterol levels. Evidence is also accumulating that a diet rich in fibre may protect against diseases associated with raised clotting factors. This investigation studied the possible effects of pectin on fibrinogen levels and fibrin network architecture. Two groups of 10 male hyperlipidaemic volunteers each, received a pectin supplement (15g/day) or placebo (15g/day) for 3 weeks. Lipid and fibrin network structure variables were measured at baseline and the end of supplementation. Pectin supplementation caused significant decreases in total cholesterol, low-density lipoprotein (a). Significant changes in the characteristics of fibrin networks developed in the

plasma of the pectin supplemented group indicated that networks were more permeable and had lower tensile strength. These network structures are believed to be less atherogenic. It is suspected that pectin modified network characteristics by a combination of its effects on metabolism and altered fibrin conversion. This confirms the therapeutic possibilities of dietary intervention. Furthermore, this study also showed that changes in plasma fibrinogen need not be present to induce alterations in fibrin network architecture.

### **Omega3 fatty acids in the prevention-management of cardiovascular disease**

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Canadian Journal of Physiology and Pharmacology (Canada), 1997, 75/3 (234-239)

Epidemiologic studies show that populations who eat fish versus those who do not have a reduced death rate from cardiovascular disease. Experimental studies have shown that omega-3 fatty acids affect the function of cells involved in atherothrombosis in numerous ways, including the modification of eicosanoid products in the cyclooxygenase and lipoxygenase pathways, the reduced synthesis of cytokines and platelet-derived growth factor, and alterations of leukocyte and endothelial cell properties. Intervention studies in patients with restenosis, myocardial infarction, and cardiac arrhythmias with omega-3 fatty acid supplementation have been addressed in several clinical studies. The ingestion of omega-3 fatty acids following one episode of myocardial infarction appears to decrease the rate of cardiac death. These effects of omega-3 fatty acids appear to be due to their antiarrhythmic properties. In fact, fish oil has been shown to reduce ventricular arrhythmias and to be more beneficial than currently used pharmacologic agents. The dose, duration, and mechanisms involved in the prevention and management of cardiovascular disease following omega-3 fatty acid ingestion or supplementation need to be investigated by double blind controlled clinical trials.

### **Vitamin intake: A possible determinant of plasma homocyst(e)ine among middle-aged adults**

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Annals of Epidemiology (USA), 1997, 7/4 (285-293)

**PURPOSE:** Many epidemiologic studies have identified elevated plasma homocyst(e)ine as a risk factor for atherosclerosis and thromboembolic diseases. To examine the relationship between vitamin intakes and plasma homocyst(e)ine,

we analyzed dietary intake data from a case-control study of 322 middle-aged individuals with atherosclerosis in the carotid artery and 318 control subjects without evidence of this disease.

**METHODS:** All of these individuals were selected from a probability sample of 15,800 men and women who participated in the Atherosclerosis Risk in Communities (ARIC) study.

**RESULTS:** Plasma homocyst(e)ine was inversely associated with intakes of folate, vitamin B6, and vitamin B12 (controls only for this vitamin)-the three key vitamins in homocyst(e)ine metabolism. Among nonusers of vitamin supplement products, on average each fertile increase in intake of these vitamins was associated with 0.4 to 0.7 micromol/L decrease in plasma homocyst(e)ine. An inverse association of plaine was also found with thiamin, riboflavin, calcium, phosphorus, and iron. Methionine and protein intake did not show any significant association with plasma homocyst(e)ine.

**CONCLUSIONS:** In almost all analyses, cases and controls showed similar associations between dietary variables and plasma homocyst(e)ine. Plasma homocyst(e)ine among users of vitamin supplement products was 1.5 micromol/L lower than that among nonusers. Further studies to examine possible caused relationships among vitamin intake, plasma homocyst(e)ine, and cardiovascular disease are needed.

### **Dietary soy protein and estrogen replacement therapy improve cardiovascular risk factors and decrease aortic cholesteryl ester content in ovariectomized cynomolgus monkeys**

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Metabolism: Clinical and Experimental (USA), 1997, 46/6 (698-705)

Estrogen replacement therapy (ERT) decreases the progression of coronary artery atherosclerosis in monkeys. Dietary soy protein also retards the progression of atherosclerosis relative to animal proteins such as casein. Soy protein contains weakly estrogenic compounds called isoflavones or phytoestrogens that may be responsible for the cardioprotective effects. This study was designed as a 2 x 2 factorial to determine the magnitude of soy protein's effects on cardiovascular risk factors relative to casein and lactalbumin, with or without estradiol treatment. Ovariectomized female monkeys were randomized to four treatment groups based on past dietary cholesterol consumption, their origin, end past reproductive history, end studied for 7 months. The animals were divided into (1) a group fed casein end lactalbumin as the protein source (n = 14), (2) a group fed casein and lactalbumin as the protein source = 13), (3) a group fed soybean protein isolate as the protein source (n = 11), and (4) a group fed soybean protein isolate as the protein source plus E2 (n = 10). Soy protein compared with casein consumption

resulted in a significant improvement in plasma lipid and lipoprotein concentrations, a significant improvement in insulin sensitivity and glucose effectiveness as determined by minimal-model analyses, and a decrease in arterial lipid peroxidation. E2-treated monkeys had a significant reduction in fasting insulin levels and insulin to glucose ratios, total body weight, and amounts of abdominal fat, and had smaller low-density lipoprotein (LDL) particles. In addition, E2 treatment resulted in a significant reduction ( $P = .001$ ) in aortic cholesteryl ester content. A similar trend ( $P = .14$ ) was found for soy protein compared with casein. There also was a significant interaction ( $P = .02$ ) with soy and E2, such that animals consuming soy protein + E2 had the least arterial cholesteryl ester content. These results suggest that both ERT and dietary soybean protein have beneficial effects on cardiovascular risk factors. Interestingly, the two treatments affected different risk factors and together resulted in the greatest reduction in arterial cholesterol content. Further studies are needed to determine the active component of the soy protein and to assess its long-term effects on the cardiovascular system and other organ systems (such as the bones and reproductive system).

### **Atherogenesis and the homocysteine-folate-cobalamin triad: Do we need standardized analyses?**

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Journal of the American College of Nutrition (USA), 1997, 16/3 (258-267)

Background: Bioscientists, physicians and nutritionists are newly interested in the homocysteine folate cobalamin triad, in part because homocysteine may be important both in atherogenesis and thrombogenesis. Homocysteine imbalance may be an early marker for cobalamin disorders because cobalamin is a cofactor in remethylation of homocysteine to methionine.

Methods: In 139 men and 32 women of similar mean age of 65 years, we measured markers which have been cited as risk for atherosclerosis: serum homocysteine, folate, total cobalamin, holotranscobalamin I and II, (TCI and TCII), total serum cholesterol (SCHOL), high density lipoprotein cholesterol (HDLC), triglycerides (STG) as well as red blood cell (RBC) folate, food records and body composition by whole body counting of potassium-40K).

Results: Statistical relationships among the data showed healthy women had lower mean serum homocysteine and their mean RBC folate and TCI and TCII were higher than men. Eighty-three subjects had TCII much lower than 60 pg/ml (subnormal), yet only 11 of these men and two women had total cobalamin <200 pg/ml (abnormal). Fifty-two subjects with serum homocysteine greater than 17.5 nmol/ml had TCII less than 60 pg/ml, suggesting serum homocysteine may be a marker for early cobalamin negative balance. None of the subjects in the study had serum folate below abnormal values, i.e., less than 1.6 mg/ml. All subjects

had RBC folate within normal range. Serum homocysteine showed inverse relationship with RBC folate and serum total cobalamin, TCI and TCII.

### **CONCLUSIONS:**

- 1) importance of using serum holotranscobalamin TCI and TCII as markers of cobalamin deficiency,
- 2) necessity to use if strong comparisons are to be made among quantitative values of serum or plasma homocysteine, folate, cobalamin, and nutrients in food intake.

### **Fasting total plasma homocysteine and atherosclerotic peripheral vascular disease**

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Annals of Vascular Surgery (USA), 1997, 11/3 (217-223)

Fasting total plasma homocysteine levels were measured by rapid ion-exchange chromatography in 100 patients with symptomatic atherosclerotic peripheral vascular disease (PVD) and 100 age and sex-matched control subjects. Demographic data, biochemistry, hematology, and lipid fractions were measured in both groups, and clinical and vascular laboratory disease parameters were recorded for the patient group. Patients with hyperhomocysteinemia (defined as those with fasting homocysteine values exceeding the 90th percentile of the control range) were compared to patients with normal homocysteine with respect to the above parameters. Total fasting homocysteine concentrations were significantly higher in the patient group (28.8 plus or minus 14.9 micromol/l) than in the control subjects (20.3 plus or minus 11.3 micromol/l;  $p < 0.001$ ). Homocysteine levels were also higher in males than in females in both the control and the patient groups. Homocysteine correlates positively only with age in the healthy controls ( $r = 0.291$ ;  $p < 0.005$ ) but not with other standard risk factors. Multivariate analysis of the biochemical risk factors confirmed that total plasma homocysteine concentration is an independent risk factor for PVD ( $p < 0.001$ ). Hyperhomocysteinemia is not associated with vitamin B12 or folate deficiency states. Vitamin B12 concentration was 591 plus or minus 313 ng/l in the control group, and 682 plus or minus 405 ng/l in the patient group ( $p = \text{NS}$ ). Serum folate concentration was lower in the controls (7.2 plus or minus 2.3 microg/l) than in the patients (8.3 plus or minus 2.0 microg/l,  $p < 0.001$ ). Mild hyperhomocysteinemia was detected in 27% of the patients. Patients with hyperhomocysteinemia has a four-fold increase in risk of PVD relative to patients with a normal homocysteine level. There is no significant difference between the two groups with respect to patient demographics, biochemical risk factors, and disease pattern and severity.

## **Plasma total homocysteine, B vitamins, and risk of coronary atherosclerosis**

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Arteriosclerosis, Thrombosis, and Vascular Biology (USA), 1997, 17/5 (989-995)

Epidemiological research has shown that elevated plasma total homocysteine (tHcy) is a risk factor for atherosclerotic disease. In the present case-control study, we investigated whether fasting or postmethionine-loading they was a stronger predictor of risk of severe coronary atherosclerosis. Furthermore, we studied levels of B vitamins, which are involved in homocysteine metabolism. Subjects were recruited from men and women, aged 25 to 65 years, who underwent coronary angiography between June 1992 and June 1994 in a hospital in Rotterdam, The Netherlands. Cases (n=131) were defined as those with less than or equal to 90% occlusion in one and less than or equal to 40% occlusion in a second coronary artery, while control subjects (n=88) had less than or equal to 50% occlusion in only one coronary vessel. In addition, a population-based control group free from clinical cardiovascular disease (n=101) was studied. Coronary patients were studied at least 2.5 months after angiography or other acute illness, such as myocardial infarction. After adjusting for age and sex differences between the groups, cases had 9% (P=.01) higher geometric mean fasting and 7% (P=.04) higher geometric mean postload they than the combined control groups. Despite higher levels of they for cases, their geometric mean levels of red cell folate and pyridoxal 5'-phosphate were higher than for control subjects, whereas plasma vitamin B12 was only slightly lower in cases. The frequency distribution of they values in cases was slightly shifted toward the right, across the entire range, compared with the distribution in the combined control group. This was somewhat more obvious for fasting than postload they levels. The odds ratio (OR) for severe coronary atherosclerosis (case status) for each 1 SD increase in fasting they (5 micromol/L) was 1.3 (95% confidence interval (CI), 1.0-1.6), similar to the OR for each 1 SD increase (12 micromol/L) in postmethionine-loading they (1.3 (95 CI, 1.0-1.7)), after adjustment for sex, age, and other potential confounders. Furthermore, there was a significant linear trend of increasing fasting they with increasing number of occluded arteries (P=.01), correcting for sex, age, and other potential confounders. Our data show a positive association between plasma they and risk of severe coronary atherosclerosis, of similar strength for fasting and postload they levels. The t that the association exists over a wide range of they levels, without a clear cutoff point below which there is no increased risk.

## **Correlation between plasma homocyst(e)ine and aortic atherosclerosis**

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American Heart Journal (USA), 1997, 133/5 (534-540)

Plasma homocyst(e)ine (H(e)) levels correlate with the prevalence of arterial occlusive diseases. Recently, transesophageal echocardiography (TEE) has been used to evaluate patients with atherosclerotic plaques in the thoracic aorta. The purpose of this study was to determine whether H(e) levels correlate with the degree of atherosclerotic plaque in the thoracic aorta (ATH) as seen on TEE. Maximum plaque areas for three locations in the thoracic aorta (arch, proximal descending, and distal descending) were measured with TEE in 156 patients. Maximum plaque areas for these locations were added to yield an estimate of ATH. ATH and H(e) levels, and levels of folic acid, vitamin B12, and pyridoxal 5'-phosphate were measured in a double-blind manner. Univariate analysis demonstrated a significant correlation of H(e) with ATH ( $r=0.3$ ,  $p < 0.001$ ). On multivariate analysis, H(e) was independently predictive of ATH ( $r$  for the model including H(e) was 0.63,  $p < 0.0001$ ). Plasma H(e) levels are therefore significantly and independently correlated with the degree of atherosclerosis in the thoracic aorta.

### **Cell cycle effects of nitric oxide on vascular smooth muscle cells**

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USA

American Journal of Physiology - Heart and Circulatory Physiology (USA), 1997, 272/4 41-4 (H1810-H1818)

We characterized the cell cycle block induced by nitric oxide (NO) on smooth muscle cells (SMC). We hypothesized that the inhibition of SMC proliferation by NO was due to a specific block in cell cycle progression. Treatment of cultured rat aortic SMC with the NO donors S-nitroso-N-acetylpenicillamine or S-nitrosoglutathione (0.1 mM for 48 h) resulted in a 50% decrease ( $P < 0.05$ ) in the fraction of cells in the S and G2+M phases and a corresponding increase in the G1 fraction, suggesting that NO inhibits entry into S phase, causing accumulation of cells in G1 phase. Application of both NO donors to cycling SMC resulted in a short-term, concentration-dependent (0.06-0.3 mM) inhibition of ongoing DNA synthesis as measured by radiothymidine incorporation, demonstrating that NO causes an S-phase arrest. The S-phase arrest by NO was not mimicked by exogenous guanosine 3',5'-cyclic monophosphate (cGMP, 10 mM) and was associated with, but not due to, a 20% inhibition of RNA synthesis. The S-phase block was completely reversed within 2 h of removal of the NO donors, similar to inhibition by the ribonucleotide reductase inhibitor hydroxyurea. Prolonged treatment of SMC with either NO donor (0.1 mM) did not synchronize cells at the G1-S boundary as expected after a prolonged S-phase arrest, but instead induced a quiescent G0-like state characterized by a 12- to 18-h lag before DNA synthesis returned to normal levels after NO removal. These findings demonstrate that NO

inhibition of SMC proliferation is associated with two distinct and reversible cell cycle arrests, an immediate cGMP-independent S-phase block followed by a shift back in the cell cycle from the G1-S boundary to a quiescent G0-like state.

### **Effects of dehydroepiandrosterone on proliferation of human aortic smooth muscle cells**

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Life Sciences (USA), 1997, 60/11 (833-838)

Dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) have association of coronary atherosclerosis in clinical and in vivo studies. However, the mechanisms responsible for the association have not been determined. In the present study, we found that DHEA influences the in vitro growth of vascular smooth muscle cells obtained from the human aorta (hASMC). The concentrations of DHEA ranging from  $10^{-8}$  M to  $10^{-6}$  M significantly stimulated the mitogenesis of hASMC in serum-free culture. On the other hand, 4 hrs of pretreatment with DHEA attenuated the fetal calf serum induced proliferative effect in a dose-dependent manner. However, the in vitro effects of DHEA on the mitogenesis observed in hASMC were not seen in rat-derived aortic smooth muscle cell lines (A10 cells). With respect to DHEAS, the hormone, at concentrations up to  $10^{-5}$  M did not affect the growth of either hASMC or A10 cells in vitro. The growth response of hASMC to DHEA in vitro was markedly affected by the culture conditions. The differential proliferative effects of DHEA on smooth muscle cells between rat and human are of interest. We conclude that the effects of DHEA on mitogenesis of hASMC may, at least in part, explain the association between DHEA and atherosclerosis.

### **Dietary fish oil: Influence on lesion regression in the porcine model of atherosclerosis**

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Arteriosclerosis, Thrombosis, and Vascular Biology (USA), 1997, 17/4 (688-694)

We examined the influence of dietary fish oil on lesion regression in a porcine model of atherogenesis. Thirty-two female Yucatan miniature pigs were fed an atherogenic diet for 8 months. A no-regression group (n=8) was killed to determine the extent of atherosclerosis at 8 months. Three regression groups were switched to normal minipig chow supplemented with either MaxEPA fish oil (FO group, n=8), a control oil with the ratio of polyunsaturated to monounsaturated to saturated fatty acid matched to that of the fish oil (CO group, n=8), or no oil supplement (NO group, n=8) for a further 4 months. Plasma cholesterol levels reached between 15 and 20 mmol/L during the atherogenic phase and returned to

normal (2 mmol/L) within 2 months of the beginning of the regression diet. Compared with the NO group, fish oil supplementation during the regression phase caused a decrease in VLDL and HDL cholesterol and an increase in LDL cholesterol. Similarly, the control oil also caused a decrease in VLDL cholesterol; however, in contrast to the FO group, HDL cholesterol increased and LDL cholesterol was unchanged. FO LDL, which had decreased levels of 20:4 (n-6 fatty acid) and increased levels of 18:3, 20:5, and 22:6 (n-3 fatty acids), was shown to be twice as susceptible to copper-mediated oxidation as CO LDL particles. Morphological examination of the major blood vessels revealed a significant reduction in lesion area in the ascending and thoracic aorta as well as the carotid artery after the regression diet; however, there was no significant difference between the fish oil and control oil groups in any of the vessels measured. Therefore, despite increased LDL, decreased HDL and an increased susceptibility to in vitro oxidation of LDL, fish oil supplementation of a regression diet did not influence lesion regression.

### **Additive hypocholesterolemic effect of psyllium and cholestyramine in the hamster: Influence on fecal sterol and bile acid profiles**

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Journal of Lipid Research (USA), 1997, 38/3 (491-502)

Recent findings suggest that the effects of cholestyramine and psyllium in combination could be additive for cholesterol-lowering. We therefore examined the effect of both agents, alone and in combination, on lipoprotein cholesterol and neutral and acidic steroid excretion in the hamster. Animals (n = 8/group) were fed for 21 days, either a basal chow diet supplemented with 10% palm oil and 0.2% cholesterol treatments consisting of the basal diet plus: 5.5% cellulose; 5% psyllium with 0.5% cellulose; 0.5% cholestyramine with 5% cellulose; or 5% psyllium with 0.5% cholestyramine. Psyllium and cholestyramine both had significant hypocholesterolemic effects, but in combination produced additive reductions in lipoprotein and hepatic cholesterol. Psyllium, cholestyramine, and the combination increased total bile acid excretion by 26%, 57%, and 79%, respectively. Psyllium affected only unconjugated bile acid excretion while cholestyramine also increased the excretion of conjugated and primary bile acids. Neither agent, nor the combination, affected fetal neutral sterol excretion. We conclude that, while both agents lower cholesterol by a mechanism of increased bile acid excretion, these studies indicate that psyllium does not bind bile acids in vivo and lend further support for the concomitant use of these agents for cholesterol-lowering.

### **Vitamin E inhibits low-density lipoprotein-induced adhesion of monocytes to human aortic endothelial cells in vitro**

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Arteriosclerosis, Thrombosis, and Vascular Biology (USA), 1997, 17/3 (429-436)

Monocyte adhesion to human aortic endothelial cells (ECs) is one of the early events in the development of atherogenesis. ECs were used to investigate the role of vitamin E in human monocyte adhesion to ECs in vitro. ECs incubated with 40 to 193 mg/dL of low-density lipoprotein cholesterol (LDL) for 22 hours exhibited increasing dose-dependent adherence for untreated, isolated human monocytes ( $P < .05$ ). ECs exposed to the highest dose of LDL (193 mg/dL) but pretreated with 19 micromol/L alpha-tocopherol for 24 hours showed a trend to lower adherence for monocytes compared with nontreated ECs (4.4 plus or minus 1.2% versus 7.6 plus or minus 1.9%;  $P = .09$ ). This effect of vitamin E became more significant ( $P < .05$ ) when ECs were exposed to a lower level of LDL (40 mg/dL) or were pretreated with a higher level of alpha-tocopherol (42 micromol/L) and then exposed to 80 mg/dL LDL. Presupplementation of ECs with 15, 19, and 37 micromol/L alpha-tocopherol significantly ( $P < .05$ ) reduced monocyte adhesion by 6 plus or minus 1%, 37 plus or minus 6%, and 69 plus or minus 17%, respectively. Levels of soluble intercellular adhesion molecule-1 (sICAM-1) molecules for monocytes, increased after incubation of ECs with LDL 80 mg/dL (4.7 plus or minus 0.7 versus 6.4 plus or minus 1.2 ng/mL, respectively;  $P < .05$ ). Treatment of ECs with alpha-tocopherol (42 micromol/L) significantly reduced induction of sICAM-1 by LDL to 2.2 plus or minus 2.3 ng/mL. After exposure to LDL, prostaglandin I<sub>2</sub> production by ECs was diminished, whereas presupplementation of ECs with alpha-tocopherol partially reversed the LDL effect. Production of interleukin-1 $\beta$  was not detectable when ECs were treated with alpha-tocopherol, LDL, or alpha-tocopherol followed by LDL. Our findings indicate that vitamin E has an inhibitory effect on LDL-induced production of adhesion molecules and adhesion of monocytes to ECs via its antioxidant function and/or its direct regulatory effect on sICAM-1 expression.

### **Nitric oxide synthase: Role in the genesis of vascular disease**

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Annual Review of Medicine (USA), 1997, 48/- (489-509)

The product of nitric oxide (NO) synthase is the most potent endogenous vasodilator known. NO not only is a potent vasodilator, it also inhibits platelet adherence and aggregation, reduces adherence of leukocytes to the endothelium, and suppresses proliferation of vascular smooth muscle cells. A number of disorders are associated with reduced synthesis and/or increased degradation of vascular NO. These include hypercholesterolemia, diabetes mellitus, hypertension, and tobacco use. The endothelial dysfunction caused by these disorders contributes to the alterations in vascular function and structure observed in these conditions. A reduction in the activity of vascular NO likely plays a significant role in the development of atherosclerosis. Insights into the

mechanisms by which NO production or activity is altered in these states will lead to new therapeutic strategies in the treatment of a number of vascular disorders, including hypertension, atherosclerosis, restenosis, and thrombosis.

### **Hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans**

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Circulation (USA), 1997, 95/5 (1119-1121)

Background: Hyperhomocyst(e)inemia is a risk factor for atherosclerosis and is prevalent in the elderly. The objective of this study was to determine whether hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans.

Methods and Results: High-resolution vascular ultrasonography was used to study endothelium-dependent and -independent vasodilation in a nonatherosclerotic peripheral conduit artery of 26 elderly hyperhomocyst(e)inemic subjects and 15 age- and sex-matched subjects with normal homocysteine levels. Flow-mediated, endothelium-dependent (nitric oxide-mediated) vasodilation was assessed by measuring the percent change in brachial artery diameter during reactive hyperemia. Endothelium-independent vasodilation was assessed after the administration of 0.4 mg sublingual nitroglycerin. Endothelium-dependent vasodilation was significantly impaired in the hyperhomocyst(e)inemic subjects compared with control subjects (3.7 plus or minus 0.6% versus 8.1 plus or minus 1.2%;  $P=0.004$ ), whereas endothelium-independent vasodilation was not different between the two groups (10.1 plus or minus 1.6% versus 9.3 plus or minus 1.5%;  $P=NS$ ). In a linear regression analysis with serum homocysteine concentration, folic acid, age, sex, cholesterol (serum total, LDL, or HDL cholesterol), mean arterial blood pressure, use of antihypertensive medication, and baseline brachial artery diameter included as covariates, serum homocysteine concentration emerged as the only significant predictor of flow-mediated vasodilation.

Conclusions: These data indicate that hyperhomocyst(e)inemia is associated with impaired endothelium-dependent vasodilation in humans and suggest that the bioavailability of nitric oxide is decreased in hyperhomocyst(e)inemic humans.

### **The role of folic acid in deficiency states and prevention of disease**

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Journal of Family Practice (USA), 1997, 44/2 (138-144)

Folic acid, a water-soluble vitamin, has been used since the 1940s to treat some cases of macrocytic anemia without neurologic disease. Folate deficiency is best diagnosed with red blood cell folate levels along with macrocytosis and/or megaloblastic anemia. In addition to reversing overt deficiency, the vitamin may reduce the incidence of neural tube defects by 45% in women who receive 400 microg per 400 microg of folate per day. Elevations in homocysteine levels, a metabolite intimately associated with folate, are also being found with increasing regularity in those with cardiovascular diseases. Homocysteine levels are reduced by folic acid administration. Therefore, there is some biologic plausibility, but not currently direct proof, for the assumption that folate supplements may prevent heart disease, stroke, and peripheral arterial disease. Controlled trials should take place before widespread food supplementation with folate is carried out on a large scale because of the possibility of outbreaks of permanent B12-related neurologic damage in those with undiagnosed pernicious anemia. However, if a patient has a premature cardiovascular event and has minimal risk factors, ordering a test to determine homocysteine level may be advisable, and if elevated, treating with folic acid supplement as long as B12 deficiency does not coexist.

### **Effects of vitamin D on aortic smooth muscle cells in culture**

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Toxicology in Vitro (United Kingdom), 1996, 10/6 (701-711)

Earlier investigations on vitamin-induced experimental atherosclerosis in rats suggested that smooth muscle cells (SMCs) play a pivotal role in development of these vascular abnormalities. This study demonstrates the effects of vitamin D (ergocalciferol) on SMCs of rat aorta in tissue culture. SMCs were obtained from aortas of newborn rats by enzymatic digestion and maintained for 6 wk in primary culture with vitamin D (1.2 nM) in the culture medium. The effects of vitamin D on SMCs, as compared with control SMCs cultures, were evaluated by light and electron microscopy. Growth of SMCs was characterized by cell counting, measurement of DNA and protein content, and by analysis of the nucleolar organizing regions. Vitamin D had no effect on proliferation of SMCs but stimulated synthesis and intercellular deposition of elastic fibres and had a stabilizing effect on the musculo-elastic multilayer formed by the cultured cells. In addition, it prevented degeneration of SMCs, with long-term preservation of the typical phenotype in primary culture.

### **Soy isoflavones enhance coronary vascular reactivity in atherosclerotic female macaques**

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Fertility and Sterility (USA), 1997, 67/1 (148-154)

Objective: To examine the effects of soy phytoestrogens on coronary vascular reactivity in atherosclerotic male and female rhesus monkeys.

Design: A prospective, randomized, blinded, controlled study.

Setting: Comparative Medicine Clinical Research Center of an academic medical center.

Patient(s): Twenty-two young adult rhesus monkeys with pre-existing diet-induced atherosclerosis. Intervention(s): Monkeys were fed soy-based diets for 6 months identical in composition, except that the isoflavones were extracted from one (low-isoflavone) and intact in the other (high- isoflavone). Quantitative coronary angiography was performed at the end of the study period. Females in the low-isoflavone group underwent a second angiography after an acute IV dose of genistein.

Main Outcome Measure(s): Percent change in diameter of the proximal left circumflex coronary artery in response to intracoronary acetylcholine and nitroglycerin, compared with control diameter.

Result(s): Arteries from males constricted in response to acetylcholine. Arteries from females in the low-isoflavone group constricted (-6.2% + 2.8%, mean plus or minus SEM), whereas arteries from females in the high- isoflavone group dilated (6.4% plus or minus 1.2%, mean plus or minus SEM). Intravenous administration of genistein caused dilation in the previously constricting low-isoflavone females (3.39% plus or minus 2.8%).

Conclusion(s): Like mammalian estrogens, dietary soy isoflavones enhance the dilator response to acetylcholine of atherosclerotic arteries in female monkeys.

### **Common mutation in methylenetetrahydrofolate reductase: Correlation with homocysteine metabolism and late-onset vascular disease**

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Circulation (USA), 1996, 94/12 (3074-3078)

Background: Increased homocysteine levels are a risk factor for atherosclerosis and its sequelae. A common genetic mutation in methylenetetrahydrofolate reductase (MTHFR), an enzyme required for efficient homocysteine metabolism, creates a thermolabile enzyme with reduced activity. We determined the

prevalence of this mutation in many subjects with and without vascular disease and related it to homocysteine and folate levels.

Methods and Results: DNA from 247 older subjects with vascular disease and 594 healthy subjects without vascular disease (in three different control groups) was screened for the MTHFR 677 C-to-T mutation. Within each group, 9% to 17% of the subjects were homozygous for this mutation, and the mutant allele frequency was 31% to 39%. The genotype distributions, homozygote frequencies, and allele frequencies did not differ significantly between the study groups. In the vascular disease subjects, despite significantly lower folate levels in MTHFR homozygotes, there was no significant difference in homocysteine levels among the MTHFR genotype groups. The negative slope of the regression line relating homocysteine and folate was significantly steeper for those with a homozygous MTHFR mutation compared with those without this mutation.

Conclusions: Although the thermolabile MTHFR mutation is very common, it does not appear to be a significant genetic risk factor for typical late-onset vascular disease. Because MTHFR homozygotes have increased homocysteine with low folate levels, this mutation may contribute to early-onset or familial vascular disease. The genotype dependence of the folate-homocysteine correlation further suggests that homozygotes for this mutation may have both an exaggerated hyperhomocysteinemic response to folic acid depletiaacid therapy.

### **Hyperhomocysteinemia confers an independent increased risk of atherosclerosis in end-stage renal disease and is closely linked to plasma folate and pyridoxine concentrations**

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Circulation (USA), 1996, 94/11 (2743-2748)

Background: A high level of total plasma homocysteine is a risk factor for atherosclerosis, which is an important cause of death in renal failure. We evaluated the role of this as a risk factor for a vascular complications of end-stage renal disease.

Methods and Results: Total fasting plasma homocysteine and other risk factors were documented in 176 dialysis patients (97 men, 79 women; mean age 56.3plus or minus14.8 years). Folate, vitamin B12, and pyridoxal phosphate concentrations were also determined. The prevalence of high total homocysteine values was determined by comparison with a normal reference population, and the risk of associated vascular complications was estimated by multiple logistic regression. Total homocysteine concentration was higher in patients than in the normal population (26.6plus or minus1.5 versus 10.1plus or minus1.7 micromol/L; P<.01). Abnormally high concentrations (>95th percentile for control subjects,

16.3 micromol/L) were seen in 149 patients (85%) with end-stage renal disease ( $P < .001$ ). Patients with a homocysteine concentration in the upper two quintiles ( $>27.8$  micromol/L) had an independent odds ratio of 2.9 (CI, 1.4 to 5.8;  $P = .007$ ) of vascular complications. B vitamin levels were lower in patients with vascular complications than in those without. Vitamin B6 deficiency was more frequent in patients than in the normal reference population versus 2%;  $P < .01$ ).

Conclusions: A high total plasma homocysteine concentration is an independent risk factor for atherosclerotic complications of end-stage renal disease. Such patients may benefit from higher doses of B vitamins than those currently recommended.

### **Dietary fats and coronary heart disease**

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Biomedicine and Pharmacotherapy (France), 1996, 50/6-7 (261-268)

The prevention and treatment of coronary heart disease (CHD) necessitates vigorous dietary intervention so as to lower the serum cholesterol level by at least 6%. Greater decreases in serum cholesterol can bring about reversal of atherosclerosis. The critical dietary change is the reduction in intake of saturated fat and cholesterol. Some of this fat may be replaced by unsaturated fats, especially monounsaturated fat (olive or canola oil). Fish and the omega-3 fats they contain may also be useful for the prevention of CHD. The benefits of omega-3 fats occur within a few months and probably involve an anti-thrombotic effect. There is evidence that the intake of trans-fatty acids formed by the hydrogenation of oils should be reduced as they are associated with CHD. Hypolipidaemic drugs may be useful for persons at very high risk of CHD but should generally be avoided for primary prevention.

### **Homocystinuria: What about mild hyperhomocysteinaemia?**

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Postgraduate Medical Journal (United Kingdom), 1996, 72/851 (513-518)

Hyperhomocysteinaemia is associated with risk of atherosclerotic vascular disease and thromboembolism, in both men and women. A variety of conditions can lead to elevated homocysteine levels, but the relation between high levels and vascular disease is present regardless of the underlying cause. Pooled data from a large number of studies demonstrate that mild hyperhomocysteinaemia after a standard methionine load is present in 21% of young patients with coronary artery disease, in 24% of patients with cerebrovascular disease, and in 32% of patients with

peripheral vascular disease. From such data an odds ratio of 13.0 (95% confidence interval 5.9 to 28.1), as an estimate of the relative risk of vascular disease at a young age, can be calculated in subjects with an abnormal response to methionine loading. Furthermore, mild hyperhomo-cysteinaemia can lead to a two- or three-fold increase in the risk of recurrent venous thrombosis. Elevated homocysteine levels can be reduced to normal in virtually all cases by simple and safe treatment with vitamin B6, folic acid, and betaine, each of which is involved in methionine metabolism. A clinically beneficial effect of such an intervention, currently under investigation, would make large-scale screening for this risk factor mandatory.

### **Effect of low dose omega-3 fatty acid supplementations on plasmalipids and lipoproteins in patients with coronary sclerosis and dyslipoproteinaemia**

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Zeitschrift für Ernährungswissenschaft (Germany), 1996, 35/2 (191-198)

In a prospective study, 20 patients (aged 48-67 years) with primary hyperlipoproteinaemia of phenotypes IIa, IIb, IV and with proven coronary sclerosis received four different doses of long-chain polyunsaturated omega-3 fatty acids. 0.18 to 1.1 g per day were administered in the form of fish oil capsules over four 2-week periods. The aim was to study the effect of different low dose supplementations of n-3 fatty acids on the plasmalipid- and lipoprotein composition and to determine a threshold of effectiveness. Significant reduction of the triglyceride level was registered in all subjects with the greatest decrease in those patients who presented with the highest base levels. The cholesterol and LDL-cholesterol values on average remained almost unchanged, apart from a significant increase of LDL-cholesterol in patients with type IV hyperlipoproteinaemia. The HDL-cholesterol fraction also showed a significant increase in type IIb patients which was related to alterations of the HDL-3 subfraction. The minimal effective dose of a daily administration of omega-3 fatty acids can be expected between 0.18 g and 0.35 g. The observed changes of plasmalipids and lipoproteins reflect the beneficial effect of saturated omega-3 fatty acids in respect to plasma-triglyceride reduction and HDL-cholesterol increase as seen in other studies, despite the use of supplementations far below 1 g per day.

### **Antioxidant of the coronary diet and disease**

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Clinica Cardiovascular (Spain), 1996, 14/2 (29-38)

High levels of cholesterol and Low Density Lipoproteins (LDL) in plasma are related to high risk to develop Coronary Heart Disease (CHD). LDL-cholesterol is a primary ingredient of the atherosclerotic plaque; its accumulation in the subendothelial space is due to peroxidative reactions. Natural antioxidants such as carotenes, polyphenolic flavonoids, vitamin E and C show defensive properties against lipid peroxidation, hence it is possible to apply these molecules in clinical therapy in the prevention of the CHD. On the other hand, alcohol, and special red wine, as well as the intake of selenium can afford a cardioprotective effect. Blood cholesterol reduction, dietary and/or due to pharmacological interventions, could modulate lipid peroxidation through a decreased production of O<sub>2</sub>·<sup>-</sup>, pivotal step in the peroxidative chain of reactions. The importance of other dietary components (fresh fruits, nuts, garlic and other vegetables as well as olive oil) have been analyzed to assess its influence and protective action in the prevention of CHD.

### **Enhanced capacity of n-3 fatty acid-enriched macrophages to oxidize low density lipoprotein mechanisms and effects of antioxidant vitamins**

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Atherosclerosis (Ireland), 1996, 124/2 (157-169)

We have investigated possible mechanisms by which n-3 fatty acid-enriched macrophages enhance the oxidation of low density lipoprotein (LDL), and the ability of antioxidant vitamins to prevent this. Macrophages were enriched with n-3 fatty acids (eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid) following incubation with fish oil. These macrophages produced large amount of TEARS in medium containing metals, and showed enhanced capacity to oxidize LDL (3-4 fold increase compared to control cells) and to accumulate the modified LDL. 5,8,11,14-eicosatetraenoic acid (ETYA, 15-lipoxygenase inhibitor) and superoxide dismutase (SOD) did not inhibit the enhanced capacity of n-3 fatty acid-enriched cells to oxidize LDL. However antioxidants, (vitamin E-enriched macrophages or vitamin C in the medium), inhibited this enhanced capacity. Medium conditioned by n-3 fatty acid-enriched cells had pro-oxidant effects on metal-initiated LDL oxidation. We conclude that n-3 fatty acid-enriched macrophages display increased oxidant capacity which is not inhibited by ETYA or SOD, and that antioxidant vitamins inhibit the enhanced capacity to oxidize LDL.

### **Prevention of preatheromatous lesions in sand rats by treatment with a nutritional supplement**

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Arzneimittel-Forschung/Drug Research (Germany), 1996, 46/6 (610-614)

Sand rats fed a hypercholesterolaemic diet containing 0.01% of the anti-thyroid agent 2-mercapto-1-imidazole develop preatheromatous lesions similar to those found in humans, in addition to obesity and insulin resistance. The effects of a nutritional supplement rich in essential fatty acids and garlic extract (Arterodiet (R)) on the appearance and evolution of the lesions were studied. Treatment with this nutritional supplement significantly decreased circulating triglycerides and low-density lipoprotein (LDL)-cholesterol levels but did not alter plasma insulin or glucose levels. Intra-arterial cholesterol levels were also decreased by the treatment which resulted in a normalisation of the atherosclerotic lesions in these animals.

### **Dietary methionine imbalance, endothelial cell dysfunction and atherosclerosis**

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Nutrition Research (USA), 1996, 16/7 (1251-1266)

Dietary factors can play a crucial role in the development of atherosclerosis. High fat, high calorie diets are well known risk factors for this disease. In addition, there is strong evidence that dietary animal proteins also can contribute to the development of atherosclerosis. Atherogenic effects of animal proteins are related, at least in part, to high levels of methionine in these proteins. An excess of dietary methionine may induce atherosclerosis by increasing plasma lipid levels and/or by contributing to endothelial cell injury or dysfunction. In addition, methionine imbalance elevates plasma/tissue homocysteine which may induce oxidative stress and injury to endothelial cells. Methionine and homocysteine metabolism is regulated by the cellular content of vitamins B6, B12, riboflavin and folic acid. Therefore, deficiencies of these vitamins may significantly influence methionine and homocysteine levels and their effects on the development of atherosclerosis.

### **Fish oil supplementation in patients with heterozygous familial hypercholesterolemia**

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Recenti Progressi in Medicina (Italy), 1996, 87/3 (102-105)

Familial hypercholesterolemia is associated with premature coronary heart disease. In patients with familial hypercholesterolemia, monotherapy with hydroxymethylglutaryl coenzyme A reductase inhibitors rarely achieves the goal of desirable low-density lipoprotein levels. Epidemiological studies suggest that populations with a high dietary intake of marine n3 fatty acids are protected against coronary heart disease. Hepatic synthesis and secretion of very low density lipoproteins are reduced during fish oil supplementation while other effects on lipid and lipoprotein metabolism are controversial. Fourteen patients affected by familial heterozygous hypercholesterolemia on chronic treatment with simvastatin were enrolled in a double blind, placebo controlled, randomized cross-over trial that evaluated the effect of fish oil ethyl ester (Esapent, 5.1 g/day) on lipid and lipoprotein serum concentrations. Total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglycerides, apoprotein B, apoprotein AI, lipoprotein (a) did not show any significant variation during the four week treatment period with fish oil ethyl ester. The present data suggest that the possible favourable influence of fish oil on the progression of atherosclerosis in these high-risk patients might involve mechanisms which are different from lipid metabolism.

### **Increased serum level of total homocysteine in CAPD patients: Despite fish oil therapy**

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Peritoneal Dialysis International (Canada), 1996, 16/Suppl. 1(S246-S249)

It has been shown that serum total homocysteine (HC) is a risk factor for vascular disease which characterizes endothelial damage. The incidence of vascular disease is increased in continuous ambulatory peritoneal dialysis (CAPD) patients. Our aim was to investigate:

- (1) whether concentration of HC correlates with atherosclerotic and inflammatory events, and
- (2) if fish oil therapy can retard the disturbance in lipid metabolism which promotes atherosclerosis.

Fourteen patients with various degrees of impaired peritoneal clearance and lipid metabolism were observed. In all patients the serum HC was elevated. Seven patients were treated with fish oil for three months. The results indicate an average increase of HC (+18%), total cholesterol (+6.6%), aggregation of erythrocytes (+9%), and an average decrease of dialysate-to-plasma creatinine (D/P) ratio (-7%), deformability of erythrocytes (-8%), and normalization of elevated soluble interleukin-2 receptor (sIL-2R) values. Regression analysis of all data demonstrated a significant correlation between HC and parameters of lipid metabolism and hemorheology. There were no significant correlations between HC and peritoneal function and serum cytokine levels. We conclude that the treatment in CAPD patients with fish oil did not improve the lipid metabolism

disturbances in atherosclerosis and peritoneal function. Elevated HC confirms the progression of the disease.

### **Metabolism of linoleic and alpha-linolenic acids in cultured cardiomyocytes: Effect of different n-6 and n-3 fatty acid supplementation**

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Molecular and Cellular Biochemistry (USA), 1996, 157/1-2 (217-222)

The metabolites of linoleic (LA) and  $\alpha$ -linolenic (ALA) acids are involved in coronary heart disease. Both n-6 and n-3 essential fatty acids (EFAs) are likely to be important in prevention of atherosclerosis since the common risk factors are associated with their reduced 6-desaturation. We previously demonstrated the ability of heart tissue to desaturate LA. In this study we examined the ability of cultured cardiomyocytes to metabolize both LA and ALA in vivo, in the absence and in the presence of gamma linolenic acid (GLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) alone or combined together. In control conditions, about 25% of LA and about 90% of ALA were converted in PUFAs. GLA supplementation had no influence on LA conversion to more unsaturated fatty acids, while the addition of n-3 fatty acids, alone or combined together, significantly decreased the formation of interconversion products from LA. Using the combination of n-6 and n-3 PUFAs, GLA seemed to counterbalance partially the inhibitory effect of EPA and DHA on LA. desaturation/elongation. The conversion of ALA to more unsaturated metabolites was greatly affected by GLA supplementation. Each supplemented fatty acid was incorporated to a significant extent into cardiomyocyte lipids, as revealed by gas chromatographic analysis. The n-6/n-3 fatty acid ratio was greatly influenced by the different supplementations; the ratio in GLA+EPA+DHA supplemented cardiomyocytes was the most similar to that recorded in control cardiomyocytes. Since important risk factors for coronary disease may be associated with reduced 6-desaturation of the parent EFAs, administration of n-6 or n-3 EFA metabolites alone could cause undesirable effects. Since they appear to have different and synergistic roles, only combined treatment with both n-6 and n-3 metabolites is likely to achieve optimum results.

### **Homocysteine, folate, and vascular disease**

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Journal of Myocardial Ischemia (USA), 1996, 8/2 (60-63)

Current evidence indicates that the genesis of atherosclerotic disease is multifactorial. One of the newly recognized factors that contributes to this process

is raised homocysteine blood levels. A variety of atherosclerotic procd by elevated homocysteine levels, including stimulation of smooth muscle cell growth, impairment of endothelial regeneration, oxidation of LDL particles, and thrombogenesis. A generic defect may account for some instances of hyperhomocysteinemia, but the majority of persons with high levels do not have known genetic defects to account for their elevations. Low levels of folic acid, vitamin B12, and pyridoxine appear to underlie most cases of elevated homocysteine levels. Adding folic acid to the diet may reduce homocysteine levels, but a link between increasing folic acid and lower risk of atherosclerotic disease has yet to be demonstrated in clinical trials. However, increasing daily folic acid intake is not unjustified in some patients. Since this may mask B12 deficiency, a supplement of cobalamin, 1 mg/d, has been proposed. In the final analysis, a clinical trial is needed to determine the true significance of hyperhomocysteinemia. Meanwhile, physicians and patients can consider increasing the daily folate intake by eating more oranges, leafy vegetables, wheat products, and cereals.

### **Nutritional interest of flavonoids**

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Medecine et Nutrition (France), 1996, 32/1 (17-27)

Polyphenols represent a complex group of compounds including several categories such as 4-oxo-flavonoids, anthocyanins and tannins. Some of these molecules are present in substantial amounts in various beverages and in plant foods (fruits, vegetables...), and several investigations have established that they were liable to cross the intestinal barrier in mammals. Significant concentrations of flavonoid or polyphenol metabolites are likely to circulate in blood plasma in humans, and it appears thus important to assess their potential biological effects. Some interesting properties have already been reported, especially as to 4-oxo-flavonoids: they have antioxidizing and metal-complexing properties, and they are liable to modulate the activity of enzymes governing important cell functions. By protecting L.D.L. from oxidative alterations and by affecting platelet functions and plasma cholesterol, flavonoids might play a protective role against atherosclerosis. Some 4-oxo-flavonoids (quercetin, genistein...) show antiproliferative properties in vitro and inhibit the development of chimio-induced cancers in animal models. Thus, together with other micronutriments, their occurrence in fruits and legumes could explain the preventive effects towards cancer risk of plant foods. Isoflavones which present a phytoestrogenic activity could be more specifically involved in the prevention of breast cancer risk. Further investigations are required to determine the actual bioavailability of the different classes of flavonoids, and to fully understand the underlying mechanisms of their biological effects.

### **The effect of reduced glomerular filtration rate on plasma total homocysteine concentration**

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Scandinavian Journal of Clinical and Laboratory Investigation (Norway), 1996,  
56/1 (41-46)

The concentration of homocysteine in plasma has been shown to be increased in renal failure, possibly contributing to the accelerated atherosclerosis observed in uraemic patients. The aim of the present study was to document the relationship between plasma total homocysteine (tHcy) concentrations and glomerular filtration rates (GFR) in highly selected patients, with renal function ranging from normal to dialysis dependency. GFR was defined as the plasma clearance of iohexol; a more accurate method than the creatinine-based estimations applied in previous studies. Plasma tHcy concentrations were highly correlated to GFR ( $r=0.70$ ,  $p<0.0001$ ) and were significantly increased already in moderate renal failure. According to a multiple regression analysis, GFR and red cell folate concentrations independently predicted plasma tHcy concentrations, whereas those of serum creatinine, plasma pyridoxal-5-phosphate, urine albumin and urine alpha-1-microglobulin (a marker of tubular damage) did not. Thus, GFR seems to be a better determinant of plasma tHcy concentration than serum creatinine concentration. Plasma total cysteine and total cysteinylglycine concentrations followed the same pattern as those of tHcy.

### **Effects of diet and exercise on qualitative and quantitative measures of LDL and its susceptibility to oxidation**

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Arteriosclerosis, Thrombosis, and Vascular Biology (USA), 1996, 16/2 (201-207)

The purpose of this study was to investigate the effects of all intensive diet and exercise program on the quantity and quality of LDL as well as its susceptibility to in vitro oxidation. The diet was low in fat (<10% kcal) and cholesterol (<100 mg/d), while high in complex, unrefined carbohydrates (>70% kcal) and fiber (35 g/1000 kcal). The study was composed of 80 participants in a 3-week residential program where food was provided ad libitum and there was daily aerobic exercise, primarily walking. In each subject, preparticipation and postparticipation fasting blood samples were drawn and LDL was isolated via density gradient ultracentrifugation. LDL particle diameter was determined by gradient gel electrophoresis of serum ( $n = 23$ ). Isolated LDL was either separated into 6 subfractions by saline gradient equilibrium ultracentrifugation ( $n=26$ ) or subjected to in vitro copper oxidation ( $n=32$ ). Significant reductions ( $P<.01$ ) in serum levels

of cholesterol (20%), LDL-cholesterol (20%), HDL-cholesterol (17%), triglycerides (26%), and glucose (16%) as well as in body weight (4%) were noted for the tibial population. The mean particle diameter of the LDL increased (242 plus or minus 0.2 to 25.1 plus or minus 0.14 nm,  $P < .01$ ) and was correlated with the reduction in serum triglycerides ( $r=58$ ,  $P<.01$ ). Six of 22 subjects changed in LDL phenotype from B (less than or equal to 25.5 nm) to A ( $>25.5$  nm). The percentage of LDL-cholesterol carried in the more dense subfractions fell significantly, while that carried by the less dense fractions increased. Initial oxidation levels fell (21%), while the lag time before copper-induced oxidation increased (13%). Reductions were observed in both the rate of oxidation (16%) and peak oxidation (20%). All of these changes should result in a dramatic reduction in the risk for atherosclerosis and its clinical sequelae.

### **Homocysteine: Relation with ischemic vascular diseases**

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Revue de Medecine Interne (France), 1996, 17/1 (34-45)

Homocysteine, a sulfur-containing amino acid, is an intermediate metabolite of methionine. Patients with homocystinuria and severe hyperhomocysteinemia develop premature arteriosclerosis and arterial thrombotic events, and venous thromboembolism. Studies suggest that moderate hyperhomocysteinemia can be considered as an independent risk factor in the development of premature cardiovascular disease. In vitro, homocysteine has toxic effects on endothelial cells. Homocysteine can promote lipid peroxidation and damage vascular endothelial cells. Moreover, homocysteine interferes with the natural anticoagulant system and the fibrinolytic system. Homocysteinemia should be known in patients with premature vascular diseases, especially in subjects with no risk factors. Folic acid, vitamin B6 can lower homocysteine levels.

### **Evaluation of hydroxyl radical-scavenging property of garlic**

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Garlic has been reported to provide protection against hypercholesterolemic atherosclerosis and ischemia-reperfusion-induced arrhythmias and infarction. Oxygen free radicals (OFRs) have been implicated as causative factors in these diseases and antioxidants have been shown to be effective against these conditions. The effectiveness of garlic in these disease states could be due to its ability to scavenge OFRs. However, the OFR-scavenging activity of garlic is not

known. Also it is not known if its activity is affected by cooking. We therefore investigated, using high pressure liquid chromatography, the ability of garlic extract (heated or unheated) to scavenge exogenously generated hydroxyl radical (.OH). .OH was generated by photolysis of H<sub>2</sub>O<sub>2</sub> (1.2-10 micromoles/ml) with ultraviolet (UV) light and was trapped with salicylic acid (500 nmoles/ml). H<sub>2</sub>O<sub>2</sub> produced .OH in a concentration-dependent manner as estimated by .OH adduct products 2,3-dihydroxybenzoic acid (DHBA) and 2,5-DHBA. Garlic extract (5 - 100 microl/ml) produced an inhibition (30 - 100%) of 2,3-DHBA and 2,5-DHBA generated by photolysis of H<sub>2</sub>O<sub>2</sub> (5.00 pmoles/ml) in concentration-dependent manner. Its activity is reduced by 10% approximately when heated to 100°C for 20, 40 or 60 min. The extent of reduction in activity was similar for the three heating periods. Garlic extract prevented the .OH-induced formation of malondialdehyde in the rabbit liver homogenate in a concentration-dependent manner. It alone did not affect the MDA levels in the absence of .OH. These results indicate that garlic extract is a powerful scavenger of .OH and that heating reduces its activity slightly.

### **Effects of interaction of RRR-alpha-tocopheryl acetate and fish oil on low-density-lipoprotein oxidation in postmenopausal women with and without hormone-replacement therapy**

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We evaluated the effects of RRR-alpha-tocopheryl acetate (alpha-tocopheryl acetate) and hormone-replacement therapy (HRT) on the oxidative susceptibility of low-density lipoprotein (LDL) in postmenopausal women consuming a fish oil supplement. The independent effect of fish oil was also assessed. Forty-eight women, equally divided in a double-blind cross over trial. Each of the four periods lasted 5 wk and was followed by a 4-wk washout interval. During each period all subjects were given a 15-g supplement of fish oil and either 0 (placebo), 100, 200, or 400 mg alpha-tocopheryl acetate daily. LDL resistance to oxidative modification was assessed by calculating lag time, propagation rate, and maximum production of conjugated dienes. Supplementation with fish oil and placebo shortened lag time and slowed propagation rate in women both using and not using HRT. After subjects consumed fish oil, supplementation with alpha-tocopheryl acetate increased plasma and LDL alpha-tocopherol contents significantly and lengthened lag time (at even the lowest concentration) but had no significant effect on propagation rate or maximum production compared with values measured after consumption of fish oil alone. Women not using HRT had faster propagation rates and higher maximum production than women using HRT; after supplementation with fish oil and alpha-tocopheryl acetate these differences prevailed. Supplements as low as 100 mg alpha-tocopheryl acetate/d increase the resistance of LDL to oxidation when fish oil supplements are used. HRT and fish oil supplements may independently affect LDL oxidative susceptibility.

## **Therapeutic actions of garlic constituents**

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Medicinal Research Reviews (USA), 1996, 16/1 (111-124)

Most studies on garlic during the past 15 years have been primarily in the fields of cardiovascular and atherosclerosis, where effects were examined on serum cholesterol, LDL, HDL, and triglycerides. Although the studies were not consistent in relation to the dosage, standardization of garlic preparations, and period of treatment, most findings suggest that garlic decreases cholesterol and triglycerides levels in patients with increased levels of these lipids. Lowering of serum lipids by garlic ingestion may decrease the atherosclerosis process. The other major beneficial effect of garlic is due to its antithrombotic actions. This field of garlic research has been extensively studied. Garlic extracts and several garlic constituents demonstrate significant antithrombotic actions both in vitro and in vivo systems. Allicin and adenosine are the most potent antiplatelet constituents of garlic because of their in vitro effects. Since both allicin and adenosine are rapidly metabolized in human blood and other tissues, it is doubtful that these compounds contribute to any antithrombotic actions in the body. In addition, ajoene also seems not to be an active antiplatelet principle, because it is not naturally present in garlic, garlic powders, or other commercial garlic preparations. Only a small amount of ajoene can be found in garlic oil-macerates; however, ajoene is being developed as a drug for treatment of thromboembolic disorders. Recent findings on the identification of potent enzyme inhibiting activities of adenosine deaminase and cyclic AMP phosphodiesterase in garlic extracts are interesting, and may have a significant role in the pharmacological actions in the body. Presence of such enzyme inhibitors in garlic may perhaps explain several clinical effects in the body, including the antithrombotic, vasodilatory, and anticancer actions. Epidemiological studies have suggested that garlic plays a significant role in the reduction of deaths caused by malignant diseases. This has led many investigators to examine garlic and garlic constituents for their antitumor and cytotoxic actions both in vitro and in laboratory animals. The data from these investigations suggest that garlic contains several potentially important agents that possess antitumor and anticarcinogenic properties. In summary, the epidemiological, clinical, and laboratory data have proved that garlic contains many biologically and pharmacologically important compounds, which are beneficial to human health from cardiovascular, neoplastic, and several other diseases. Numerous studies are in progress all over the world to develop effective and odorless garlic preparations, as well as to isolate the active principles that may be therapeutically useful.

## **Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys**

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Although the beneficial effects of dietary soybean protein compared with animal proteins on plasma lipids, lipoproteins and atherosclerosis have been known for about 50 years, it has been uncertain whether these effects are due to its amino acid concentrations or other components in soybeans. To assess the effect of soybean protein's alcohol-extractable components (including the isoflavonic phytoestrogens genistein and daidzein) on plasma lipid and lipoprotein concentrations and to test fed 27 peripubertal male and female rhesus monkeys moderately atherogenic diets in which the source of dietary protein was a soy isolate (20% by weight), either containing phytoestrogens (also termed isoflavones) or with the phytoestrogens removed by alcohol extraction. The study was a crossover design with each period lasting for 6 mo. The phytoestrogen-intact soy protein (compared with the alcohol-extracted soy protein) had favorable effects on plasma lipid and lipoprotein concentrations, specifically by significantly reducing LDL + VLDL cholesterol concentrations in both males and females (similar 30-40% lower), significantly increasing high density lipoprotein cholesterol (HDL-C) concentrations for females (similar 15% higher) and significantly lowering total plasma cholesterol (TPC):HDL-C ratios (similar 20% lower for males and 50% lower for females). The phytoestrogens had no adverse effects on the reproductive systems of either the males or females, as evaluated by reproductive hormone concentrations and organ weights at necropsy. Thus, the isoflavones in soy protein improve cardiovascular disease risk factors without apparent deleterious effects on the reproductive system of peripubertal rhesus monkeys.

### **High dose B-vitamin treatment of hyperhomocysteinemia in dialysis patients**

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Kidney International (USA), 1996, 49/1 (147-152)

Hyperhomocysteinemia, an arteriosclerotic risk factor, persists in 75% of dialysis patients despite routine low dose supplementation with the B-vitamin co-factors/substrates for homocysteine (Hcy) metabolism, and normal or supernormal plasma status of these vitamins (Atherosclerosis 114:93, 1995). We conducted a placebo-controlled eight-week trial of the effect on plasma homocysteine of adding supraphysiologic dose folic acid (15 mg/day), B-6 (100 mg/day), and B-12 (1 mg/day) to the usual daily dosing of 1 mg folic acid, 10 mg B-6, and 12 microg B-12, in 27 hyperhomocysteinemic dialysis patients. Total plasma homocysteine was measured at baseline, and after four and eight weeks. Blinded analyses revealed no evidence of toxicity in the group randomized to

supraphysiologic dose B-vitamin supplementation. Plasma homocysteine was significantly reduced after both four weeks (-29.8% vs. -2.0%;  $P = 0.0024$ ) and eight weeks (-25.8% vs. +0.6%;  $P = 0.0009$ ) of active versus placebo treatment. Also, 5 of 15 treated versus 0 of 12 placebo group patients had their plasma Hcy reduced to within the normative range ( $< 15$  micromol/liter). Supraphysiologic doses of B-vitamins may be required to correct hyperhomocystiduria.

### **Long-term folic acid (but not pyridoxine) supplementation lowers elevated plasma homocysteine level in chronic renal failure**

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Mineral and Electrolyte Metabolism (Switzerland), 1996, 22/1-3 (106-109)

Moderate hyperhomocysteinemia, a risk factor for premature atherosclerosis, is present in chronic uremic patients. We prospectively evaluated the effects of sequential supplementation with pyridoxine (70 mg/day) and folic acid (10 mg/day) for two 3-month periods in 37 nondialyzed patients (29 males) with creatinine clearance (C(Cr)) ranging from 10 to 80 ml/min, whose plasma vitamin B12 and folate level was in the normal range. Mean (plus or minus SD) baseline plasma total homocysteine (Hcy) was 14.9 plus or minus 5.2, 16.5 plus or minus 5.1 and 26.1 plus or minus 12.1 micromol/l (upper limit in 45 healthy controls 14.1 micromol/l) in patients with C(Cr) 40-80, 20-40 and  $< 20$  ml/min, respectively. Following pyridoxine Hcy did not significantly decrease whereas following folic acid Hcy decreased significantly to 9.9 plus or minus 2.9 (-33% vs. baseline), 10.3 plus or minus 3.4 (-37%) and 15.4 plus or minus 5.5 (-40%), respectively (Student's paired t test,  $p < 0.001$ ) in the 3 groups. We conclude that folate (but not pyridoxine) pharmacologic supplementation is effective in lowering elevated plasma Hcy in chronic renal failure patients, thus suggesting that enhancing the Hcy remethylation pathway may overcome hyperhomocysteinemia in such patients. In view of the potential atherogenic effects of hyperhomocysteinemia, long-term folate supplementation should be considered in uremic patients.

### **Ascorbate and urate are the strongest determinants of plasma antioxidative capacity and serum lipid resistance to oxidation in Finnish men**

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Copper-induced plasma lipoprotein oxidation resistance has usually been determined in density lipoprotein (LDL) fractions, that do not contain water-soluble

antioxidants present in blood plasma. The aim of this study was to find the main determinants of the measurements of copper-induced lipid oxidation (lag time) in whole serum and plasma total peroxyl radical trapping capacity (TRAP) in a population sample of smoking (n = 25) or non-smoking (n = 26) middle aged men at high risk of cardiovascular diseases. Smokers had significantly lower plasma ascorbic acid values, but only slightly lower alpha-tocopherol, beta-carotene and serum urate values than non-smokers. Plasma ascorbic acid concentration explained 23.5% of the lag time variation (standardized regression coefficient  $\beta = 0.48$ ;  $P = 0.004$ ) in smokers and 5.6% in non-smokers. Serum urate concentration was the strongest determinant of lag time in non-smokers ( $\beta = 0.64$ ,  $P < 0.001$ ). In addition, serum albumin, lipid standardized alpha-tocopherol and serum high density lipoprotein (HDL) cholesterol entered the multivariate regression model for lag time. For plasma TRAP, only urate and ascorbic acid entered the multivariate regression model. Lag times in serum and in isolated very low density lipoprotein (VLDL) and LDL fraction did not correlate, but the maximal rate of these reactions correlated significantly. These results confirm that lipid peroxidation resistance in serum or plasma are associated with ascorbic acid, urate, alpha-tocopherol, albumin and HDL concentrations. The measurement of lipid oxidation resistance in whole serum might be more physiological than in isolated lipoprotein fraction, as the effects of water-soluble antioxidants are not artificially removed.

### **Antioxidants in the prevention of atherosclerosis**

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Current Opinion in Lipidology (United Kingdom), 1996, 7/6 (374-380)

Four antioxidant treatment for heart disease are scrutinized: probucol, beta-carotene, alpha-tocopherol and anti-iron treatment. A pattern seems to have emerged in which some treatments look promising, but others are disappointing. Most published studies of antioxidation in atherosclerosis have been ad-hoc in that the primary endpoint of the study has not been a diagnosis related to atherosclerosis; this may be misleading. The most promising antioxidant seems to be alpha-tocopherol, supported by the results of the Cambridge Heart Antioxidant Study. Probuco has the drawback of decreasing high density lipoprotein concentration and is therefore unlikely to influence atheroma in people. beta-Carotene has been repeatedly shown to be ineffective against coronary heart disease. Anti-iron treatment has not yet been tested in animal models or in man. More has to be learned of the role of antioxidation in atherosclerosis before the effectiveness of this treatment modality can be established.

### **The carotenoids beta-carotene, canthaxanthin and zeaxanthin inhibit macrophage-mediated LDL oxidation**

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FEBS Letters (Netherlands), 1997, 401/2-3 (262-266)

Human monocyte-macrophages were incubated for 24 h in Ham's F-10 medium with human low-density lipoprotein (LDL) in the presence or absence of beta-carotene, canthaxanthin or zeaxanthin, at final concentrations of 2.5, 12.5 and 25 mg/l. LDL oxidation, measured by agarose gel electrophoresis, the thecarotenoids in a concentration-dependent manner. Canthaxanthin was more effective when incorporated into LDL before addition to the cultures whereas beta-carotene and zeaxanthin were more effective when added simultaneously with LDL. The results suggest that dietary carotenoids might help slow atherosclerosis progression.

### **Polyunsaturated fatty acids, antioxidants, and cognitive function in very old men**

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Atherosclerosis and thrombosis may lead to cognitive impairment through cerebral infarcts or white matter hyperintensities. Oxidative stress is now seen as a major contributor to the process of atherogenesis. High intake of polyunsaturated fatty acids, e.g., linoleic acid, or low intake of antioxidants can increase oxidative stress. High intake of n-3 polyunsaturated fatty acids and its main source, fish, may reduce the risk of thrombosis. Little is known, however, about the relation between these dietary factors and cognitive function. The authors investigated this relation with data derived from a cohort of men, aged 69-89 years, who were participants in the Zutphen Elderly Study. The 30-point Mini-Mental State Examination was used to assess cognitive impairment in 1990 (score less than or equal to 25 in 153/476 men, 32%) and cognitive decline from 1990 to 1993 (drop >2 points in 51/342 men, 15%). Food intake was estimated in 1985 and 1990 by the cross-check dietary history method. High linoleic acid intake was associated with cognitive impairment after adjustment for age, education, cigarette smoking, alcohol consumption, and energy intake (odds ratio (OR) for highest vs. lowest tertile = 1.76, 95% confidence interval (CI) 1.04-3.01). Intake of n-3 polyunsaturated fatty acids was not associated with cognitive impairment, whereas high fish consumption tended to be inversely associated with cognitive impairment (OR = 0.63, 95% CI 0.33-1.21) and cognitive decline (OR = 0.45, 95% CI 0.17-1.16). Intakes of beta-carotene, vitamins C and E, and flavonoids were not inversely associated with cognitive impairment or decline. This study raises the possibility that high linoleic acid intake is positively associated with cognitive impairment and high fish consumption inversely associated with cognitive impairment.

### **Animal studies on antioxidants**

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Journal of Cardiovascular Risk (United Kingdom), 1996, 3/4 (358-362)

The wealth of existing epidemiologic evidence suggests that antioxidant intake limits the clinical expression of coronary artery disease. Because the oxidative modification of low-density lipoprotein is an important event in atherogenesis, it has been attractive to speculate that antioxidants act by limiting low-density lipoprotein oxidation and, as a consequence, atherosclerotic lesion development. Early studies on animals also suggested that a number of structurally distinct antioxidant compounds could limit the extent of lesion development in animal models of atherosclerosis. More recently, however, secure evidence linking the antioxidant protection of low-density lipoprotein with a reduction in atherosclerosis has been elusive. This discrepancy may be explained by emerging evidence demonstrating that antioxidants may prove beneficial through tissue-specific effects that are not strictly related to the antioxidant protection of low-density lipoprotein.

### **Alpha-Tocopherol and beta-carotene serum levels in post-menopausal women treated with transdermal estradiol and oral medroxyprogesterone acetate**

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Hormone and Metabolic Research (Germany), 1996, 28/10 (558-561)

Estrogens exert a protective effect against atherosclerosis. It is well known that hormone replacement therapy (HRT) can effectively decrease LDL-cholesterol and increase HDL-cholesterol and Apo-AI serum levels. Some recent studies have suggested that estrogens alone or in association with progestins may exert an antioxidant effect on lipids. Besides sex steroids, also vitamins exert an antioxidant effect on LDL and may preserve the endogenous antioxidants of LDL. The aim of our study was to evaluate whether HRT can improve alpha-tocopherol and beta-carotene serum levels in post-menopausal women. Fifteen postmenopausal women with climacteric symptoms were treated with 50 microg/24 h estradiol transdermally applied twice a week for 21 days. A daily dose of 10 mg oral medroxyprogesterone acetate was added for 12 days in each treatment cycle. This therapy lasted 6 months. A significant reduction was found in total cholesterol and LDL-cholesterol after treatment. Besides, our study has shown that alpha-toc/LDL and beta-car/LDL ratios significantly increased after treatment, while alpha-tocopherol and beta-carotene serum levels did not change

significantly after therapy. These preliminary findings suggest that HRT can preserve the content of alpha-tocopherol and beta-carotene in LDL.

### **Antioxidant status of hypercholesterolemic patients treated with LDL apheresis**

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Cardiovascular Drugs and Therapy (USA), 1996, 10/5 (567-571)

Oxidation of low density lipoprotein is involved in the pathogenesis of atherosclerosis. Epidemiological studies suggest a negative correlation between the occurrence of cardiovascular diseases and blood concentrations of lipophilic antioxidants such as vitamins A and E and beta-carotene. Trace elements, such as zymes glutathione peroxidase and superoxide dismutase. The aim of this study was to determine the antioxidant and trace element status of patients with severe hypercholesterolemia who had been treated with dextran-sulphate low-density lipoprotein apheresis in comparison with two control populations, normocholesterolemic subjects and untreated hypercholesterolemic patients. Our results showed that, patients treated with LDL apheresis, compared with normocholesterolemic subjects, were not deficient in vitamin E, beta-carotene, and copper, but had low plasma levels of selenium, zinc, and vitamin A. The low selenium and vitamin A levels were due to the LDL apheresis treatment, and the hypercholesterolemia might have provoked the low plasma levels of zinc. This study pointed out the potential benefits of supplemental selenium, zinc, and vitamin A in patients being treated with LDL apheresis.

### **Abnormal antioxidant vitamin and carotenoid status in chronic renal failure**

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Oxidative modification of plasma lipoproteins increases their atherogenicity. Nutritive antioxidants, including carotenoids, can prevent such lipoperoxidation and may protect against atherosclerosis. Plasma retinol, ascorbate, alpha-tocopherol and four carotenoids (lutein, lycopene, alpha-carotene and beta-carotene) were measured using HPLC in 45 patients with chronic renal failure (CRF) and in 21 controls. Plasma retinol was significantly increased in patients with CRF (conservative therapy mean of 3.7micromol/l vs. 1.9micromol/l;  $p <$

0.001). Plasma lycopene was significantly lower in patients with CRF (healthy mean 0.44 micromol/l vs. conservative therapy mean 0.27 micromol/l and haemodialysis mean of 0.17 micromol/l;  $p < 0.001$ ), a finding that persisted even after adjusting for plasma cholesterol. Low circulating antioxidant lycopene levels may contribute to an already impaired antioxidant defence system in patients with CRF. The process of haemodialysis further compromises antioxidant defences, principally by removing water-soluble ascorbate and urate, but does not appear to affect circulating carotenoid concentrations.

### **Antioxidants in cardiovascular disease: Randomized trials**

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Nutrition (USA), 1996, 12/9 (583-588)

The hypothesis that antioxidant vitamins might reduce cardiovascular disease risk is based on a large body of both basic and human epidemiologic research. One of the most consistent findings in dietary research is that those who consume higher amounts of fruits and vegetables have lower rates of heart disease and stroke as well as cancer. Recent attention has focused on the antioxidant content of fruits and vegetables as a possible explanation for the apparent protective effects. Basic research provides a plausible mechanism by which antioxidants might reduce the risk of atherosclerosis. A large number of descriptive, case-control and cohort studies provide data suggesting that consumption of antioxidant vitamins is associated with reduced risks of cardiovascular disease. These data raise the question of a possible role of antioxidants, such as vitamins C and E, and beta carotene, in the primary prevention of cardiovascular disease but do not provide a definitive answer. Results from several large-scale randomized trials of antioxidant supplements are now available; however, results are not entirely consistent. The results of the major trials do not prove or disprove the value of antioxidant vitamins, nor do they incriminate them as harmful. They do, however, raise the possibility that some of the benefits from observational epidemiology may have been overestimated and that there may be some adverse effects. At this point randomized trial data are not yet sufficient to fully assess the risk-to-benefit ratios for antioxidant supplements. More reliable data should be forthcoming in the near future which will better define the role of antioxidants in the primary and secondary prevention of atherosclerotic disease as well as cancer.

### **Dietary antioxidants and cognitive function in a population-based sample of older persons: The Rotterdam study**

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American Journal of Epidemiology (USA), 1996, 144/3 (275-280)

Antioxidants have been implicated in processes related to atherosclerosis, aging, and selective neuronal damage, all of which may ultimately affect cognitive function. In a sample of older persons, the authors examined the cross-sectional relation between cognitive function and dietary intake of beta-carotene and vitamins C and E. The data were derived from 5,182 community participants aged 55-95 years in the population-based Rotterdam Study in the period 1990 to 1993. Dietary intake was estimated from a semi-quantitative food frequency questionnaire and categorized into five levels of intake. Cognitive function was measured with the 30-point Mini- Mental State Examination (MMSE) and characterized as unimpaired ( $>25$  points) or impaired (less than or equal to 25 points). Logistic regression analysis was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for cognitive impairment. After adjustment for age, education, sex, smoking, total caloric intake, and intake of other antioxidants, a lower intake of beta-carotene was associated with impaired cognitive function ( $<0.9$  mg vs. less than or equal to 2.1 mg intake, OR = 1.9, 95% CI 1.2-3.1;  $p$  for trend  $< 0.04$ ). There was no association between cognitive function and intake of vitamins C and E. These cross-sectional observations are compatible with the view that beta-carotene-rich foods may protect against cognitive impairment in older people. The finding could also reflect unmeasured confounding, measurement error, or a change in food habits that resulted from rather than preceded the onset of cognitive impairment.

### **Lack of correlation between the alpha-tocopherol content of plasma and LDL, but high correlations for gamma-tocopherol and carotenoids**

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Journal of Lipid Research (USA), 1996, 37/9 (1936-1946)

In 59 healthy human subjects (37 male and 22 female) the concentrations of the lipid-soluble antioxidants alpha and gamma-tocopherol, alpha- and beta-carotene, lycopene, cryptoxanthin, canthaxanthin, and lutein + zeaxanthin were determined in plasma (micromol/L) and in isolated low density lipoproteins (LDL) (micromol/mmol cholesterol). Plasma alpha-tocopherol concentrations were significantly correlated with plasma total cholesterol concentrations ( $r^2 = 0.51$ ,  $P < 0.0001$ ) yet not with the LDL alpha-tocopherol content ( $r^2 = 0.05$ , ns). Plasma gamma-tocopherol concentrations were weakly correlated with plasma total cholesterol ( $r^2 = 0.12$ ,  $P < 0.003$ ) and both absolute and cholesterol standardized plasma gamma-tocopherol concentrations correlated strongly with the LDL gamma-tocopherol content ( $r^2 = 0.58$  and  $r^2 = 0.72$ , respectively). In contrast, carotenoid concentrations did not correlate with cholesterol concentrations, but their LDL content correlated significantly with the respective plasma concentrations ( $r^2 = 0.67$  to  $0.92$ , all  $P < 0.0001$ ). In a subgroup of study subjects

(n = 13) the distribution of vitamin E and carotenoids among LDL was calculated. The proportion of plasma alpha- and gamma- tocopherol found in LDL was 48 plus or minus 7 (range, 36-61%) and 41 plus or minus 7%, respectively, suggesting that LDL was in most of these subjects not the main carrier for these antioxidants. The lipophilic carotenoids, however, were predominantly carried by LDL (e.g., beta-carotene: 87 plus or minus 10%), whereas the proportion of the more polar ones carried by LDL was much smaller (e.g., lutein + zeaxanthin: 36 plus or minus 6%). The results of this study show that plasma alpha-tocopherol concentrations are not predictive for the alpha-tocopherol content of LDL in nonsupplemented individuals. This finding could have implications in interpreting the cause of the inverse relationship between plasma alpha- tocopherol and risk of atherosclerosis.

### **Oxidized low density lipoproteins in atherogenesis: Role of dietary modification**

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Annual Review of Nutrition (USA), 1996, 16/- (51-71)

The development of atherosclerosis is a complex and multistep process. There are many determinants in the pathogenesis of this condition, with different factors presumably playing key roles at different times in the evolution of the atherosclerotic plaque. It has been suggested that oxidation of low density lipoproteins (LDL) by cells in the artery wall leads to a proatherogenic particle that may help initiate early lesion formation. For this reason, understanding the determinants of LDL susceptibility to oxidation is essential for developing therapeutic strategies to inhibit this process. Oxidation of LDL begins with the abstraction of hydrogen from polyunsaturated fatty acids; thus, LDL fatty acid composition undoubtedly contributes to the process of LDL oxidation. Since dietary fatty acids influence the fatty acid composition of LDL and cell membranes, the amount and type of fat in the diet may effect susceptibility of LDL and cells to oxidative damage. Additionally, since cell membrane fatty acid composition also influences cellular formation of reactive oxygen species, dietary fatty acids may help determine the prooxidant activity of artery wall cells. Both cells and lipoproteins contain a variety of antioxidants that provide protection against oxidative stress. A major source of these antioxidants is the diet. Enrichment of the diet with foods high in such antioxidants as vitamin E, beta-carotene, or vitamin C, or supplementation of the diet with antioxidant vitamins, may inhibit oxidation and the process of atherosclerosis.

### **Effect of dietary supplementation of beta-carotene on human monocyte - macrophage-mediated oxidation of low density lipoprotein**

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Israel Journal of Medical Sciences (Israel), 1996, 32/6 (473-478)

Oxidative modification of low density lipoprotein (LDL), a key step in early atherosclerosis, is protected by the lipoprotein-associated antioxidants. The present study analyzes the effect of beta-carotene in plasma, in LDL and in monocyte-macrophages, on macrophage-mediated oxidation of LDL. We investigated the effect of dietary supplementation of beta-carotene on plasma lipid peroxidation (induced by AAPH (2,2-Azobis-2-amidinopropane hydrochloride)] and on cell-free and cell-mediated oxidation of LDL by human monocyte-derived macrophages (HMDM) in the presence of CuSO<sub>4</sub>. Significant enrichment with beta-carotene was noted in plasma (twofold), in LDL (2.6-fold) and in HMDM (1.6-fold) 2 weeks after dietary supplementation with 180 mg/day of beta-carotene. Plasma lipid peroxidation analyzed by conjugated dienes generation decreased by 22% ( $P < 0.01$ ) and LDL susceptibility to oxidation analyzed by malondialdehyde generation decreased by 40% ( $P < 0.01$ ). After beta-carotene supplementation, beta-carotene-enrichment of HMDM did not affect HMDM capacity to oxidize native LDL, whereas beta-carotene enrichment of LDL significantly reduced LDL oxidation. In conclusion, then, our results suggest that beta-carotene content of LDL, but not that of the macrophages, is responsible for the inhibition of oxidation of LDL.

### **Increased oxidation resistance of atherogenic plasma lipoproteins at high vitamin E levels in non-vitamin E supplemented men**

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Atherosclerosis (Ireland), 1996, 124/1 (83-94)

The oxidative modification of human low density lipoprotein (LDL) has been widely investigated. However, there are no very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and low density lipoprotein fraction, although all of them are atherogenic and contain antioxidants such as alpha-tocopherol. We investigated the oxidation susceptibility and oxidation resistance of VLDL + LDL (including IDL) fraction by induction with CuCl<sub>2</sub> and its relation to plasma alpha-tocopherol concentration and lipid standardised alpha-tocopherol concentration in 406 non-vitamin E-supplemented men from eastern Finland. Even though we did not give oral vitamin E or any other antioxidant supplementation to our study participants, we observed a significant, consistent relationship between measurements of oxidation resistance and plasma content of vitamin E. In the multivariate regression model, a high plasma content of vitamin E or lipid standardised vitamin E concentration were the most important determinants of lag time to maximal oxidation rate (standardised regression coefficient = 0.244,  $P < 0.0001$  for vitamin E and 0.211,  $P < 0.0001$  for lipid standardised vitamin E). After statistical adjustment for age, use of cigarettes, hypolipidemic medication (yes vs.

no), month of the measurements, plasma concentrations of total ascorbic acid (ascorbic acid + dehydroascorbic acid), beta-carotene and phospholipids, serum concentrations of LDL cholesterol and triglycerides and dietary intake of linoleic acid, the lag time to maximal oxidation rate was 10% (95% C.I. 6.0-13.5%) longer in men in the highest fifth than in the lowest fifth of plasma vitamin E content ( $P < 0.0001$  for trend). When the fifths of lipid standardised vitamin E were compared, the lag time to maximal oxidation rate was 6% (95% C.I. 1.8-10.1%) longer in men in the highest than in the lowest fifth ( $P < 0.0001$  for trend). Our data suggest that alpha-tocopherol is an important antioxidant preventing the in vitro oxidation of VLDL + LDL fraction even in non-supplemented subjects.

### **Increased levels of autoantibodies to cardiolipin and oxidised low density lipoprotein are inversely associated with plasma vitamin C status in cigarette smokers**

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*Atherosclerosis (Ireland)*, 1996, 124/1 (75-81)

In this study we have measured circulating levels of autoantibodies to cardiolipin and oxidised low-density lipoprotein (ox-LDL) and correlated these with plasma concentrations of the anti-oxidant nutrients vitamin C, vitamin E and beta-carotene, in a group (79) of asymptomatic, male cigarette smokers and in non-smoking control subjects. Cigarette smoking, a well-known risk factor for development of atherosclerosis, was found to be associated with moderately elevated levels of autoantibodies to both cardiolipin and ox-LDL. Increased levels of these autoantibodies were most evident in the older smokers ( $> 30$  years) and were significantly and inversely correlated with plasma vitamin C, but not with vitamin E or beta-carotene. Absorption studies designed to investigate the specificity of these autoantibodies demonstrated a high degree of cross-reactivity of cardiolipin antibodies with ox-LDL, while antibodies to the oxidatively modified lipoprotein tended to be specific for this antigen. These findings suggest that cigarette smoking promotes formation of autoantibodies to both cardiolipin and ox-LDL and that these may be involved in the initiation and/or perpetuation of atherosclerosis. Dietary intake of vitamin C may be a determinant of susceptibility to development of this cardiovascular disorder.

### **Antioxidant vitamins and risk of cardiovascular diseases**

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Sang, thrombose, vaisseaux: *STV*, Vol. 8, No. 4, p. 210

In recent years, an increasing number of basic and clinical research reports have pointed out the role of reactive metabolites of oxygen, the free radicals, in the processes of atherogenesis and also the protective and/or preventive effects of antioxidant molecules such as beta-carotene, vitamin C, vitamin E, selenium and zinc. Epidemiological data obtained by cross-sectional, case-control and prospective studies also provide strong supportive arguments in favour of the relationship between intake of antioxidant vitamins (or biological status in antioxidant vitamins) and cardiovascular risk. However, the epidemiological nature of the studies does not enable confirmation of a causal role. Cause-effect relationships would require controlled intervention trials of antioxidant vitamins versus placebo to assess potential effects on cardiovascular morbidity and mortality.

### **Nutritional supplement program halts progression of early coronary atherosclerosis documented by ultrafast computed tomography**

Rath M.; Niedzwiecki A.

USA

Journal of Applied Nutrition (USA), 1996, 48/3 (68-78)

The aim of this study was to determine the effect of a defined nutritional supplement program on the natural progression of coronary artery disease. This nutritional supplement program was composed of vitamins, amino acids, minerals, and trace elements, including a combination of essential nutrients patented for use in the prevention and reversal of cardiovascular disease. The study was designed as a prospective intervention before-after trial over a 12 month period and included 55 patients (age 44-67) with various stages of coronary heart disease. Changes in the progression of coronary artery calcification before and during the nutritional supplement intervention were determined by Ultrafast Computed Tomography (Ultrafast CT). The natural progression rate of coronary artery calcification before the intervention averaged 44% per year. The progression of coronary artery calcification decreased on average 15% over the course of one year of nutritional supplementation. In a sub-group of patients with early stages of coronary artery disease, a statistically significant decrease occurred, and no further progression of coronary calcification was observed. In individual cases, reversal and complete disappearance of previously existing coronary calcifications were documented. This is the first clinical study documenting the effectiveness of a defined nutritional supplement program in halting early forms of coronary artery disease within one year. The nutritional supplement program tested here should be considered an effective and safe approach to prevention and adjunct therapy of cardiovascular disease.

### **Metal excretion and magnesium retention in patients with intermittent claudication treated with intravenous disodium EDTA**

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Department of Surgery, Hillerod Hospital, Denmark.  
Clin Chem 1996 Dec;42(12):1938-42

Sixty patients with intermittent claudication participated in a double-blind placebo-controlled trial of 20 courses of intravenous chelation therapy with 3 g of disodium EDTA vs placebo during 5-9 weeks. After the first infusion, the 24-h urinary excretion of lead and zinc was similar 25-fold higher in the EDTA-treated group; relative differences for copper and calcium were smaller. Urinary magnesium excretion in the EDTA-treated group was one-third less than in the control group. After the treatment period, the blood lead concentration had decreased by similar 73% and the serum zinc concentration by similar 34%; other changes in blood concentrations were negligible. The loss of essential minerals and the possible redistribution of lead in the body may constitute a disadvantage that should be taken into account in repeated intravenous EDTA treatment.

## 8. Attention Deficit Hyperactivity Disorder

Preventative and curative options include:

Vitamins B and C, zinc, magnesium, choline, DMAE, glutamine, GABA, DHEA, phosphatidylserine, fish oil, ginkgo, ginseng, theanine.

### **Alternative treatments for adults with attention-deficit hyperactivity disorder.**

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Ann N Y Acad Sci 2001 Jun;931:310-41

A previous review of alternative treatments (Tx) of ADHD--those other than psychoactive medication and behavioral/psychosocial Tx--was supplemented with an ADDitional literature search focused on adults with ADHD. Twenty-four alternative Tx were identified, ranging in scientific documentation from discrediting controlled studies through mere hypotheses to positive controlled double-blind clinical trials. Many of them are applicable only to a specific subgroup. Although oligoantigenic (few-foods) diets have convincing double-blind evidence of efficacy for a properly selected subgroup of children, they do not appear promising for adults. Enzyme-potentiated desensitization, relaxation/EMG biofeedback, and deleading also have controlled evidence of efficacy. Iron supplementation, magnesium supplementation, Chinese herbals, EEG biofeedback, massage, meditation, mirror feedback, channel-specific perceptual training, and vestibular stimulation all have promising prospective pilot data, many of these tests reasonably controlled. Single-vitamin megadosage has some intriguing pilot trial data. Zinc supplementation is hypothetically supported by systematic case-control data, but no systematic clinical trial. Laser acupuncture has promising unpublished pilot data and may be more applicable to adults than children. Essential fatty acid supplementation has promising systematic case-control data, but clinical trials are equivocal. RDA vitamin supplementation, non-Chinese herbals, homeopathic remedies, and antifungal therapy have no systematic data in ADHD. Megadose multivitamin combinations are probably ineffective for most patients and are possibly dangerous. Simple sugar restriction seems ineffective. Amino acid supplementation is mildly effective in the short term, but not beyond 2-3 months. Thyroid treatment is effective in the presence of documented thyroid abnormality. Some alternative Tx of ADHD are effective or probably effective, but mainly for certain patients. In some cases, they are the Tx of choice, and initial evaluation should consider the relevant etiologies. A few have failed to prove effective in controlled trials. Most need research to determine whether they are effective and/or to define the applicable subgroup. Some of them, although not safer than standard Tx, may be preferable for an etiologic subgroup.

## **Does zinc moderate essential fatty acid and amphetamine treatment of attention-deficit/hyperactivity disorder?**

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J Child Adolesc Psychopharmacol 2000 SUMMMER;10(2):111-7

Zinc is an important co-factor for metabolism relevant to neurotransmitters, fatty acids, prostaglandins, and melatonin, and indirectly affects dopamine metabolism, believed intimately involved in attention-deficit/hyperactivity disorder (ADHD). To explore the relationship of zinc nutrition to essential fatty acid supplement and stimulant effects in treatment of ADHD, we re-analyzed data from an 18-subject double-blind, placebo-controlled crossover treatment comparison of d-amphetamine and Efamol (evening primrose oil, rich in gamma-linolenic acid). Subjects were categorized as zinc-adequate (n = 5), borderline zinc (n = 5), and zinc-deficient (n = 8) by hair, red cell, and urine zinc levels; for each category, placebo-active difference means were calculated on teachers' ratings. Placebo-controlled d-amphetamine response appeared linear with zinc nutrition, but the relationship of Efamol response to zinc appeared U-shaped; Efamol benefit was evident only with borderline zinc. Placebo-controlled effect size (Cohen's d) for both treatments ranged up to 1.5 for borderline zinc and dropped to 0.3-0.7 with mild zinc deficiency. If upheld by prospective research, this post-hoc exploration suggests that zinc nutrition may be important for treatment of ADHD even by pharmacotherapy, and if Efamol benefits ADHD, it likely does so by improving or compensating for borderline zinc nutrition.

## **Relationships between serum-free fatty acids and zinc, and attention deficit hyperactivity disorder: a research note.**

Bekaroglu M, Aslan Y, Gedik Y, Deger O, Mocan H, Erduran E, Karahan C. Department of Psychiatry, Technical University, Faculty of Medicine, Trabzon, Turkey.

J Child Psychol Psychiatry 1996 Feb;37(2):225-7

The purpose of this study is to evaluate the relationships between serum free fatty acids (FFA) and zinc, and attention deficit hyperactivity disorder (ADHD). Forty eight children with ADHD (33 boys, 15 girls) were included in the patient group and 45 healthy volunteer children (30 boys, 15 girls) constituted the control group. The mean serum FFA level in the patient group was 0.176 +/- 0.102 mEq/L and in control group, 0.562 +/- 0.225 mEq/L (< .001). The mean serum zinc level of patient group was 60.6 +/- 9.9 micrograms/dl and that of the control group, 105.8 +/- 13.2 micrograms/dl (< .001). A statistically significant correlation was found between zinc and FFA levels in the ADHD group. These findings indicate that zinc deficiency may play a role in aetiopathogenesis of ADHD. Although we observed decreased FFA levels in ADHD cases, it is necessary to determine whether this condition is a principal cause of ADHD or is secondary to zinc deficiency.

### **Attention deficit and infantile hyperactivity.**

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Rev Enferm 2001 Jan;24(1):11-4

Hyperactivity is a very common disorder in children (specially males) that today is considered as a clinical syndrome by scientific medicine. American Psychiatric Association establishes 10 symptoms to diagnose it, but they can be resumed in three characteristics: Impulsivity, Distraction, and Hyperactivity. There are different ways to treat it, but psychiatric medication has major risks in children. From complementary medicine we can find several aids in changing diet patterns and supplementing with vitamins or minerals. Chocolate, sugar, sweeteners, additives, preservatives, dyes, can enhance the incidence of this syndrome; instead the supplementation with lipids rich in PUFA's can prevent it. B complex vitamins, magnesium, copper, manganese or calcium can be interesting and in herbal medicine, sedative plants like passion flower, valerian or lemon balm are useful aids. Also liquorice, fennel and berries can be used for different physiological actions.

### **The effect of pyridoxine hydrochloride on blood serotonin and pyridoxal phosphate contents in hyperactive children.**

Bhagavan HN, Coleman M, Coursin DB

Pediatrics 1975 Mar;55(3):437-41

The contents of serotonin (hydroxytryptamine) and pyridoxal phosphate (PLP) in the blood of 11 hyperactive children and 11 controls were determined on an outpatient basis. A significant decrease in serotonin content was found in blood samples from hyperactive patients as compared with controls. There were no differences in PLP content of blood between the two groups. Four children were selected for a study of the effects of pyridoxine hydrochloride (vitamin B6) on low serotonin levels. Oral doses of pyridoxine resulted in an appreciable increase in the serotonin content and a very large increase in the PLP content of blood in these hyperactive patients.

### **Long-chain polyunsaturated fatty acids in children with attention-deficit hyperactivity disorder.**

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Am J Clin Nutr 2000 Jan;71(1 Suppl):327S-30S

Attention-deficit hyperactivity disorder (ADHD) is the diagnosis used to describe children who are inattentive, impulsive, and hyperactive. ADHD is a widespread condition that is of public health concern. In most children with ADHD the cause

is unknown, but is thought to be biological and multifactorial. Several previous studies indicated that some physical symptoms reported in ADHD are similar to symptoms observed in essential fatty acid (EFA) deficiency in animals and humans deprived of EFAs. We reported previously that a subgroup of ADHD subjects reporting many symptoms indicative of EFA acid than did ADHD subjects with few such symptoms or control subjects. In another study using deficiency (L-ADHD) had significantly lower proportions of plasma arachidonic acid and docosahexaenoic contrast analysis of the plasma polar lipid data, subjects with lower compositions of total n-3 fatty acids had significantly more behavioral problems, temper tantrums, and learning, health, and sleep problems than did those with high proportions of n-3 fatty acids. The reasons for the lower proportions of long-chain polyunsaturated fatty acids (LCPUFAs) in these children are not clear; however, factors involving fatty acid intake, conversion of EFAs to LCPUFA products, and enhanced metabolism are discussed. The relation between LCPUFA status and the behavior problems that the children exhibited is also unclear. We are currently testing this relation in a double-blind, placebo-controlled intervention in a population of children with clinically diagnosed ADHD who exhibit symptoms of EFA deficiency.

**On the role of cortical glutamate in obsessive-compulsive disorder and attention-deficit hyperactivity disorder, two phenomenologically antithetical conditions.**

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Acta Psychiatr Scand. 2000 Dec;102(6):401-13.

**OBJECTIVE:** The objective of the present study was to compare the phenomenology and pathophysiology of obsessive-compulsive disorder (OCD) and attention-deficit hyperactivity disorder/deficits in attention, motor control and perception (ADHD/DAMP).

**METHOD:** Through detailed studies of the literature on OCD and ADHD/DAMP the phenomenology of these two conditions is compared, and possible underlying pathophysiological mechanisms involving interactions between glutamate, dopamine, serotonin and acetylcholine are discussed, with emphasis on OCD. The present paper also discusses possible mechanisms of action for current pharmacological treatments of OCD and ADHD, as well as possible future treatment strategies for these disorders.

**RESULTS:** OCD and ADHD/DAMP are common neuropsychiatric conditions which in many regards appear to be each other's antipodes with respect to clinical manifestations, associated personality traits and brain biochemistry, notably prefrontal cortical glutamate activity. Future pharmacological treatments of these disorders may involve manipulations with glutamate, dopamine D1, serotonin 2A and nicotine receptors.

CONCLUSION: It appears that OCD is a hyperglutamatergic and ADHD a hypoglutamatergic condition, with prefrontal brain regions being especially affected.

**The influence of soy-derived phosphatidylserine on cognition in age-associated memory impairment.**

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Nutr Neurosci 2001;4(2):121-34

Phosphatidylserine (PS) is a phospholipid widely sold as a nutritional supplement. PS has been claimed to enhance neuronal membrane function and hence cognitive function, especially in the elderly. We report the results of a clinical trial of soybean-derived PS (S-PS) in aging subjects with memory complaints. Subjects were 120 elderly (< 57 years) of both sexes who fulfilled the more stringent criteria for age-associated memory impairment (AAMI); some also fulfilled the criteria for age-associated cognitive decline. Subjects were allocated at random to one of the three treatment groups: placebo, 300mg S-PS daily, or 600mg S-PS daily. Assessments were carried out at baseline, after 6 and 12 weeks of treatment, and after a wash-out period of 3 weeks. Tests of learning and memory, choice reaction time, planning and attentional functions were administered at each assessment. Delayed recall and recognition of a previously learned word list comprised the primary outcome measures. No significant differences were found in any of the outcome variables between the treatment groups. There were also no significant interactions between treatment and 'severity of memory complaints'. In conclusion, a daily supplement of S-PS does not affect memory or other cognitive functions in older individuals with memory complaints.

**Attention deficit/hyperactivity disorder (ADHD) in children: rationale for its integrative management.**

Kidd PM.

Altern Med Rev 2000 Oct;5(5):402-28

Attention Deficit/Hyperactivity Disorder (ADHD) is the most common behavioral disorder in children. ADHD is characterized by attention deficit, impulsivity, and sometimes overactivity ("hyperactivity"). The diagnosis is empirical, with no objective confirmation available to date from laboratory measures. ADHD begins in childhood and often persists into adulthood. The exact etiology is unknown; genetics plays a role, but major etiologic contributors also include adverse responses to food additives, intolerances to foods, sensitivities to environmental chemicals, molds, and fungi, and exposures to neurodevelopmental toxins such as heavy metals and organohalide pollutants. Thyroid hypofunction may be a common denominator linking toxic insults with ADHD symptomatology.

Abnormalities in the frontostriatal brain circuitry and possible hypofunctioning of dopaminergic pathways are apparent in ADHD, and are consistent with the benefits obtained in some instances by the use of methylphenidate (Ritalin) and other potent psychostimulants. Mounting controversy over the widespread use of methylphenidate and possible life-threatening effects from its long-term use make it imperative that alternative modalities be implemented for ADHD management. Nutrient deficiencies are common in ADHD; supplementation with minerals, the B vitamins (added in singly), omega-3 and omega-6 essential fatty acids, flavonoids, and the essential phospholipid phosphatidylserine (PS) can ameliorate ADHD symptoms. When individually managed with supplementation, dietary modification, detoxification, correction of intestinal dysbiosis, and other features of a wholistic/integrative program of management, the ADHD subject can lead a normal and productive life.

### **Herbs of activating blood circulation to remove blood stasis.**

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Clin Hemorheol Microcirc 2000;23(2-4):127-31

Drugs with the efficacy of modifying rheological properties of blood, blood vessels and their interactions are denoted by "hemorheologicals". Drugs of anti-hyperviscosemia, anti-coagulants, anti-platelet drugs, anti-thrombotics, vasodilators, endothelial cell protectors and anti-arthrosclerosis should be considered as hemorheologicals due to the actions in keeping blood fluidity and in maintaining normal vascular functions. The studies in hemorheology indicate that a tendency of hyperviscosity, hypercoagulation and being prone to thrombosis is prevalent in the elderly. Hemorheologicals are importance for and aging and life-threatening diseases. Blood stasis syndrome is a common pathological syndrome in the elderly. In traditional Chinese medicine, the treatment for the syndrome is by herbs which activates blood circulation to remove blood stasis. The herbs have the efficacy of improving hemorheological events. Therefore, the herbs are the source for developing hemorheologicals. Ligustrazine isolated from Chuangxiong is an example. It showed significant inhibition on shear induced platelet aggregation and on platelet intracellular calcium demonstrated by laser confocal microscope.

### **Effect of the herbal extract combination *Panax quinquefolium* and *Ginkgo biloba* on attention-deficit hyperactivity disorder: a pilot study.**

Lyon MR, Cline JC, Totosy de Zepetnek J, Shan JJ, Pang P, Benishin C. Oceanside Functional Medicine Research Institute, Nanaimo, BC.

J Psychiatry Neurosci 2001 May;26(3):221-8

OBJECTIVE: A combination herbal product containing American ginseng extract, *Panax quinquefolium*, (200 mg) and *Ginkgo biloba* extract (50 mg) (AD-

FX; CV Technologies, Edmonton, Alta.) was tested for its ability to improve the symptoms of attention-deficit hyperactivity disorder (ADHD).

DESIGN: Open study.

PATIENTS: 36 children ranging in age from 3 to 17 years who fit the diagnostic criteria for ADHD.

INTERVENTIONS: AD-FX capsules were taken twice a day on an empty stomach for 4 weeks. Patients were instructed not to change any other medications during the study.

OUTCOME MEASURES: At the beginning of the study, after 2 weeks, and then at the end of the 4-week trial, parents completed the Conners' Parent Rating Scale-revised, long version, a questionnaire that assesses a broad range of problem behaviours (and was used as an indication of ADHD symptom severity).

RESULTS: After 2 weeks of treatment, the proportion of the subjects exhibiting improvement (i.e., decrease in T-score of at least 5 points) ranged from 31% for the anxious-shy attribute to 67% for the psychosomatic attribute. After 4 weeks of treatment, the proportion of subjects exhibiting improvement ranged from 44% for the social problems attribute to 74% for the Conners' ADHD index and the DSM-IV hyperactive-impulsive attribute. Five (14%) of 36 subjects reported adverse events, only 2 of which were considered related to the study medication.

CONCLUSIONS: These preliminary results suggest AD-FX treatment may improve symptoms of ADHD and should encourage further research on the use of ginseng and Ginkgo biloba extracts to treat ADHD symptoms.

### **The potential role of fatty acids in attention-deficit/hyperactivity disorder.**

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Prostaglandins Leukot Essent Fatty Acids 2000 Jul-Aug;63(1-2):79-87

As currently defined, attention-deficit/hyperactivity disorder (ADHD) encompasses a broad constellation of behavioural and learning problems and its definition and diagnosis remain controversial. The aetiology of ADHD is acknowledged to be both complex and multifactorial. The proposal considered here is that at least some features of ADHD may reflect an underlying abnormality of fatty acid metabolism. Clinical and biochemical evidence is discussed which suggests that a functional deficiency of certain long-chain polyunsaturated fatty acids could contribute to many of the features associated with this condition. The implications in terms of fatty acid treatment proposals are also discussed; such a form of treatment is relatively safe compared to existing pharmacological interventions, although further studies are still needed in order to evaluate its potential efficacy in the management of ADHD symptoms. Copyright 2000 Harcourt Publishers Ltd.

**A randomized double-blind, placebo-controlled study of the effects of supplementation with highly unsaturated fatty acids on ADHD-related symptoms in children with specific learning difficulties.**

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Prog Neuropsychopharmacol Biol Psychiatry 2002 Feb;26(2):233-9

(1) The authors tested the prediction that relative deficiencies in highly unsaturated fatty acids (HUFAs) may underlie some of the behavioral and learning problems associated with attention-deficit/hyperactivity disorder (ADHD) by studying the effects of HUFA supplementation on ADHD-related symptoms in children with specific learning difficulties (mainly dyslexia) who also showed ADHD features. (2) Forty-one children aged 8-12 years with both specific learning difficulties and above-average ADHD ratings were randomly allocated to HUFA supplementation or placebo for 12 weeks. (3) At both baseline and follow-up, a range of behavioral and learning problems associated with ADHD was assessed using standardized parent rating scales. (4) At baseline, the groups did not differ, but after 12 weeks mean scores for cognitive problems and general behavior problems were significantly lower for the group treated with HUFA than for the placebo group; there were significant improvements from baseline on 7 out of 14 scales for active treatment, compared with none for placebo. Group differences in change scores all favored HUFA, reaching conventional significance levels for 3 out of 14 scales. (5) HUFA supplementation appears to reduce ADHD-related symptoms in children with specific learning difficulties. Given the safety and tolerability of this simple treatment, results from this pilot study strongly support the case for further investigations.

**The effect of vitamin-mineral supplementation on juvenile delinquency among American schoolchildren: a randomized, double-blind placebo-controlled trial.**

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J Altern Complement Med 2000 Feb;6(1):7-17

CONTEXT: Numerous studies conducted in juvenile correctional institutions have reported that violence and serious antisocial behavior have been cut almost in half after implementing nutrient-dense diets that are consistent with the World Health Organization's guidelines for fats, sugar, starches, and protein ratios. Two controlled trials tested whether the cause of the behavioral improvements was psychological or biological in nature by comparing the behavior of offenders who either received placebos or vitamin-mineral supplements designed to provide the micronutrient equivalent of a well-balanced diet. These randomized trials reported that institutionalized offenders, aged 13 to 17 years or 18 to 26 years, when given active tablets produced about 40% less violent and other antisocial behavior than

the placebo controls. However, generalization could not be made to typical schoolchildren without a controlled trial examining violence and antisocial behavior in public schools.

**OBJECTIVES:** To determine if schoolchildren, aged 6 to 12 years, who are given low dose vitamin-mineral tablets will produce significantly less violence and antisocial behavior in school than classmates who are given placebos.

**DESIGN:** A stratified randomized, double-blind, placebo-controlled trial with pretest and post-test measures of antisocial behavior on school property.

**SETTINGS AND SUBJECTS:** Two "working class," primarily Hispanic elementary schools in Phoenix, Arizona. Approximately half of the potential schoolchildren participated, i.e., 468 students aged 6 to 12 years.

**INTERVENTION:** Daily vitamin-mineral supplementation at 50% of the U.S. recommended daily allowance (RDA) for 4 months versus placebo. The supplement was designed to raise vitamin-mineral intake up to the levels currently recommended by the National Academy of Sciences for children aged 6 to 11 years.

**OUTCOME MEASURE:** Violent and nonviolent delinquency as measured by official school disciplinary records.

**RESULTS:** Of the 468 students randomly assigned to active or placebo tablets, the 80 who were disciplined at least once between September 1st and May 1st served as the research sample. During intervention, the 40 children who received active tablets were disciplined, on average, 1 time each, a 47% lower mean rate of antisocial behavior than the 1.875 times each for the 40 children who received placebos (95% confidence interval, 29% to 65%,  $< 5 .020$ ). The children who took active tablets produced lower rates of antisocial behavior in 8 types of recorded infractions: threats/fighting, vandalism, being disrespectful, disorderly conduct, defiance, obscenities, refusal to work or serve, endangering others, and nonspecified offenses.

**CONCLUSION:** Poor nutritional habits in children that lead to low concentrations of water-soluble vitamins in blood, impair brain function and subsequently cause violence and other serious antisocial behavior. Correction of nutrient intake, either through a well-balanced diet or low-dose vitamin-mineral supplementation, corrects the low concentrations of vitamins in blood, improves brain function and subsequently lowers institutional violence and antisocial behavior by almost half. This paper adds to the literature by enabling previous research to be generalized from older incarcerated subjects with a history of antisocial behavior to a normal population of younger children in an educational setting.

**The effects of magnesium physiological supplementation on hyperactivity in children with attention deficit hyperactivity disorder (ADHD). Positive response to magnesium oral loading test.**

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Magnes Res 1997 Jun;10(2):149-56

Children with ADHD are 'a group at risk' as far as their further emotional and social development and educational possibilities are concerned, and the consequences of the lack of an appropriate therapy appears to be serious. Some of these children do not respond to prevailing therapy methods. It is reported that dietetic factors can play a significant role in the etiology of ADHD syndrome, and magnesium deficiency can help in revealing hyperactivity in children. The aim of our work was to assess the influence of magnesium supplementation on hyperactivity in patients with ADHD. The examination comprised 50 hyperactive children, aged 7-12 years, who fulfilled DSM IV criteria for ADHD syndrome, with recognized deficiency of magnesium in the blood (blood serum and red blood cells) and in hair using atomic absorption spectroscopy. In the period of 6 months those examined regularly took magnesium preparations in a dose of about 200 mg/day. 30 of those examined with ADHD showed coexisting disorders specific to developmental age, and 20 of them showed disruptive behaviour. The control group consisted of 25 children with ADHD and magnesium deficiency, who were treated in a standard way, without magnesium preparations. 15 members of this group showed coexisting disorders specific for developmental age, and 10 members showed disruptive behaviour. Hyperactivity was assessed with the aid of psychometric scales: the Conners Rating Scale for Parents and Teachers, Wender's Scale of Behavior and the Quotient of Development to Freedom from Distractibility. In the group of children given 6 months of magnesium supplementation, independently of other mental disorders coexisting with hyperactivity, an increase in magnesium contents in hair and a significant decrease of hyperactivity of those examined has been achieved, compared to their clinical state before supplementation and compared to the control group which had not been treated with magnesium.

### **Is attention-deficit/hyperactivity disorder an energy deficiency syndrome?**

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Biol Psychiatry 2001 Aug 1;50(3):151-8

Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable yet clinically heterogeneous syndrome associated with hypocatecholamine function in subcortical and prefrontal cortical regions and clinical response to medications that enhance catecholamine function. The goal of this article is to present a hypothesis about the etiology of ADHD by synthesizing these findings with recent experiments indicating that activity-dependent neuronal energy consumption is regulated by cortical astrocytes. The scientific literature was searched from 1966 to the present using MEDLINE and relevant key words. Inattention and impulsivity may be related to hypofunctionality of catecholamine projection pathways to prefrontal cortical areas, resulting in decreased neuronal

energy availability. This may be mediated by astrocyte catecholamine receptors that normally regulate energy availability during neuronal activation. At least some forms of ADHD may be viewed as cortical, energy-deficit syndromes secondary to catecholamine-mediated hypofunctionality of astrocyte glucose and glycogen metabolism, which provides activity-dependent energy to cortical neurons. Several tests of this hypothesis are proposed.

### **Spirulina maxima prevents induction of fatty liver by carbon tetrachloride in the rat.**

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Biochem Mol Biol Int. 1998 Apr;44(4):787-93

The aim of the present work was to assess the capacity of *Spirulina maxima* to prevent fatty liver development induced in rats by an intraperitoneal single dose (1 ml/kg) of carbon tetrachloride. Liver and serum lipids were quantified two or four days after treatment with this agent. Liver lipid concentration did not differ in rats fed on a purified diet with or without *Spirulina*. However, after carbon tetrachloride treatment, liver triacylglycerols were significantly lower in rats fed on a diet with *Spirulina* 5% than in rats without *Spirulina* in their diet ( $< 0.05$ ). Furthermore, the increased liver cholesterol values, induced by carbon tetrachloride treatment, were not observed in rats that received *Spirulina*. These results support the potential hepatoprotective role of *Spirulina*.

### **A randomized, double-blind, placebo-controlled trial of docosahexanoic acid supplementation in children with attention-deficit/hyperactivity disorder.**

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J Pediatr. 2001 Aug;139(2):189-96.

**OBJECTIVE:** To determine whether docosahexanoic acid (DHA) supplementation for 4 months decreases the symptoms of attention-deficit/hyperactivity disorder (ADHD).

**STUDY DESIGN:** Sixty-three 6- to-12-year-old children with ADHD, all receiving effective maintenance therapy with stimulant medication, were assigned randomly, in a double-blind fashion, to receive DHA supplementation (345 mg/d) or placebo for 4 months. Outcome variables included plasma phospholipid fatty acid patterns, scores on laboratory measures of inattention and impulsivity (Test of Variables of Attention, Children's Color Trails test) while not taking stimulant medication, and scores on parental behavioral rating scales (Child Behavior Checklist, Conners' Rating Scale). Differences between groups after 4 months of DHA supplementation or placebo administration were determined by analysis of

variance, controlling for age, baseline value of each outcome variable, ethnicity, and ADHD subtype.

**RESULTS:** Plasma phospholipid DHA content of the DHA-supplemented group was 2.6-fold higher at the end of the study than that of the placebo group (4.85 +/- 1.35 vs 1.86 +/- 0.87 mol % of total fatty acids; < 001). Despite this, there was no statistically significant improvement in any objective or subjective measure of ADHD symptoms.

**CONCLUSION:** A 4-month period of DHA supplementation (345 mg/d) does not decrease symptoms of ADHD.