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## Journal ABSTRACTS

### Vitamin D

#### EPIDEMIC INFLUENZA AND VITAMIN D.

In 1981, R. Edgar Hope-Simpson proposed that a 'seasonal stimulus' intimately associated with solar radiation explained the remarkable seasonality of epidemic influenza. Solar radiation triggers robust seasonal vitamin D production in the skin; vitamin D deficiency is common in the winter, and activated vitamin D, 1,25(OH)<sub>2</sub>D, a steroid hormone, has profound effects on human immunity. 1,25(OH)<sub>2</sub>D acts as an immune system modulator, preventing excessive expression of inflammatory cytokines and increasing the 'oxidative burst' potential of macrophages. Perhaps most importantly, it dramatically stimulates the expression of potent anti-microbial peptides, which exist in neutrophils, monocytes, natural killer cells, and in epithelial cells lining the respiratory tract where they play a major role in protecting the lung from infection. Volunteers inoculated with live attenuated influenza virus are more likely to develop fever and serological evidence of an immune response in the winter. Vitamin D deficiency predisposes children to respiratory infections. Ultraviolet radiation (either from artificial sources or from sunlight) reduces the incidence of viral respiratory infections, as does cod liver oil (which contains vitamin D). An interventional study showed that vitamin D reduces the incidence of respiratory infections in children. We conclude that vitamin D, or lack of it, may be Hope-Simpson's 'seasonal stimulus'.

*Epidemiol Infect.* 2006 Dec;134(6):1129-40

#### PROINFLAMMATORY CYTOKINE RESPONSES INDUCED BY INFLUENZA A (H5N1) VIRUSES IN PRIMARY HUMAN ALVEOLAR AND BRONCHIAL EPITHELIAL CELLS.

**BACKGROUND:** Fatal human respiratory disease associated with influenza A subtype H5N1 has been documented in Hong Kong, and more recently in Vietnam, Thailand and Cambodia. We previously demonstrated that patients with H5N1 disease had unusually high serum levels of IP-10 (interferon-gamma-inducible protein-10). Furthermore, when compared with human influenza virus subtype H1N1, the H5N1 viruses in 1997 (A/Hong Kong/483/97) (H5N1/97) were more potent inducers of pro-inflammatory cytokines (e.g. tumor necrosis factor- $\alpha$ ) and chemokines (e.g. IP-10) from primary human macrophages in vitro, which suggests that cytokines dysregulation may play a role in pathogenesis of H5N1 disease. Since respiratory epithelial cells are the primary target cell for replication of influenza viruses, it is pertinent to investigate the cytokine induction profile of H5N1 viruses in these cells. **METHODS:** We used quantitative RT-PCR and ELISA to compare the profile of cytokine and chemokine gene expression induced by H5N1 viruses A/HK/483/97 (H5N1/97), A/Vietnam/1194/04 and A/Vietnam/3046/04 (both H5N1/04) with that of human H1N1 virus in human primary alveolar and bronchial epithelial cells in vitro. **RESULTS:** We demonstrated that in comparison to human H1N1 viruses, H5N1/97 and H5N1/04 viruses were more potent inducers of IP-10, interferon beta, RANTES (regulated on activation, normal T cell expressed and secreted) and interleukin 6 (IL-6) in primary human alveolar and bronchial epithelial cells in vitro. Recent H5N1 viruses from Vietnam (H5N1/04) appeared to be even more potent at inducing IP-10 than H5N1/97 virus. **CONCLUSION:** The H5N1/97 and H5N1/04 subtype influenza A viruses are more potent inducers of proinflammatory cytokines and chemokines in primary human respiratory epithelial cells than subtype H1N1 virus. We suggest that this hyper-induction of cytokines may be relevant to the pathogenesis of human H5N1 disease.

*Respir Res.* 2005 Nov 11;6:135

#### TOLL-LIKE RECEPTOR TRIGGERING OF A VITAMIN D-MEDIATED HUMAN ANTIMICROBIAL RESPONSE.

In innate immune responses, activation of Toll-like receptors (TLRs) triggers direct antimicrobial activity against intracellular bacteria, which in murine, but not human, monocytes and macrophages is mediated principally by nitric oxide. We report here that TLR activation of human macrophages up-regulated expression of the vitamin D receptor and the vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular *Mycobacterium tuberculosis*. We also observed that sera from African-American individuals, known to have increased susceptibility to tuberculosis, had low 25-hydroxyvitamin D and were inefficient in supporting cathelicidin messenger RNA induction. These data support a link between TLRs and vitamin D-mediated innate immunity and suggest that differences in ability of human populations to produce vitamin D may contribute to susceptibility to microbial infection.

### **ANTIMICROBIAL PEPTIDES IN THE AIRWAY.**

The airway provides numerous defense mechanisms to prevent microbial colonization by the large numbers of bacteria and viruses present in ambient air. An important component of this defense is the antimicrobial peptides and proteins present in the airway surface fluid (ASF), the mucin-rich fluid covering the respiratory epithelium. These include larger proteins such as lysozyme and lactoferrin, as well as the cationic defensin and cathelicidin peptides. While some of these peptides, such as human beta-defensin (hBD)-1, are present constitutively, others, including hBD2 and -3 are inducible in response to bacterial recognition by Toll-like receptor-mediated pathways. These peptides can act as microbicides in the ASF, but also exhibit other activities, including potent chemotactic activity for cells of the innate and adaptive immune systems, suggesting they play a complex role in the host defense of the airway. Inhibition of antimicrobial peptide activity or gene expression can result in increased susceptibility to infections. This has been observed with cystic fibrosis (CF), where the CF phenotype leads to reduced antimicrobial capacity of peptides in the airway. Pathogenic virulence factors can inhibit defensin gene expression, as can environmental factors such as air pollution. Such an interference can result in infections by airway-specific pathogens including *Bordetella bronchiseptica*, *Mycobacterium tuberculosis*, and influenza virus. Research into the modulation of peptide gene expression in animal models, as well as the optimization of peptide-based therapeutics shows promise for the treatment and prevention of airway infectious diseases.

*Curr Top Microbiol Immunol.* 2006;306:153-82

### **DEFECTS IN THE SYNTHESIS AND METABOLISM OF VITAMIN D.**

It is now recognized that it is casual exposure to sunlight that provides most humans with their vitamin D requirement. During exposure to sunlight, the high energy ultraviolet B photons (290-315 nm) photolyzes cutaneous stores of 7-dehydrocholesterol to previtamin D<sub>3</sub>. Once formed, previtamin D<sub>3</sub> undergoes a thermal isomerization that results in the formation of vitamin D<sub>3</sub>. Vitamin D<sub>3</sub> is biologically inert and requires successive hydroxylations in the liver and kidney to form its biologically active hormone 1,25-dihydroxy-vitamin D<sub>3</sub>. The major physiologic function of 1,25-dihydroxy-vitamin D<sub>3</sub> is to maintain blood calcium in the normal range. It accomplishes this by increasing the efficiency of intestinal calcium absorption and mobilizing stem cells to become osteoclasts which, in turn, remove calcium from the bone. It is now recognized that there are a variety of calcium metabolic disorders that are related to defects in the synthesis and metabolism of vitamin D. Chronic granulomatous disorders are often associated with hypercalciuria and hypercalcemia. The mechanism by which this occurs is that activated macrophages within granulomatous tissue, in an unregulated manner, convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. Besides its calcemic activity 1,25-dihydroxyvitamin D<sub>3</sub> is a potent antiproliferative factor for cells and tissues that possess its vitamin D receptor. This has clinical utility in that 1,25-dihydroxyvitamin D<sub>3</sub> and its analogs have been successfully used for the treatment of the hyperproliferative skin disease psoriasis.

*Exp Clin Endocrinol Diabetes.* 1995;103(4):219-27

### **THE ROLE OF VITAMIN D FOR BONE HEALTH AND FRACTURE PREVENTION.**

Vitamin D inadequacy is pandemic in adults. Vitamin D deficiency causes osteopenia, precipitates and exacerbates osteoporosis, causes the painful bone disease osteomalacia, and increases muscle weakness, which worsens the risk of falls and fractures. Vitamin D deficiency can be prevented by sensible sun exposure and adequate supplementation. Monitoring serum 25-hydroxyvitamin D is the only way to determine vitamin D status. Recent recommendations suggest that in the absence of sun exposure, adults should ingest 1000 IU of vitamin D<sub>3</sub> per day. The ideal healthy blood level of 25-hydroxyvitamin D should be 30 to 60 ng/mL. Vitamin D intoxication occurs when 25-hydroxyvitamin D levels are greater than 150 ng/mL. Three recent reports suggesting that vitamin D and calcium supplementation does not decrease the risk of fracture will be put into perspective in light of the vast literature supporting increasing vitamin D and calcium intake as an effective method for decreasing risk of vertebral and nonvertebral fractures.

*Curr Osteoporos Rep.* 2006 Sep;4(3):96-102

### **CALCIUM AND VITAMIN D NUTRITION AND BONE DISEASE OF THE ELDERLY.**

Osteoporosis, a systemic skeletal disease characterized by a low bone mass, is a major public health problem in EC member states because of the high incidence of fragility fractures, especially hip and vertebral fracture. In EC member states the high incidence of osteoporotic fractures leads to considerable mortality, morbidity, reduced mobility and decreased quality of life. In 1995 the number of hip fractures in 15 countries of EC has been 382,000 and the estimated total care cost of about 9 billion of ECUs. Given the magnitude of the problem public health measures are important for preventive intervention. Skeletal bone mass is determined by a combination of endogenous (genetic, hormonal) and exogenous (nutritional, physical activity) factors. Nutrition

plays an important role in bone health. The two nutrients essential for bone health are calcium and vitamin D. Reduced supplies of calcium are associated with a reduced bone mass and osteoporosis, whereas a chronic and severe vitamin D deficiency leads to osteomalacia, a metabolic bone disease characterized by a decreased mineralization of bone. Vitamin D insufficiency, the preclinical phase of vitamin D deficiency, is most commonly found in the elderly. The major causes of vitamin D deficiency and insufficiency are decreased renal hydroxylation of vitamin D, poor nutrition, scarce exposition to sunlight and a decline in the synthesis of vitamin D in the skin. The daily average calcium intake in Europe has been evaluated in the SENECA study concerning the diet of elderly people from 19 towns of 10 European countries. In about one third of subjects the dietary calcium intake results were very low, between 300 and 600 mg/day in women, and 350 and 700 mg/day in men. Calcium supplements reduce the rate of bone loss in osteoporotic patients. Some recent studies have reported a significant positive effect of calcium treatment not only on bone mass but also on fracture incidence. The SENECA study, has also shown that vitamin D insufficiency is frequent in elderly populations in Europe. There are a number of studies on the effects of vitamin D supplementation on bone loss in the elderly, showing that supplementations with daily doses of 400-800 IU of vitamin D, given alone or in combination with calcium, are able to reverse vitamin D insufficiency, to prevent bone loss and to improve bone density in the elderly. In recent years, there has been much uncertainty about the intake of calcium for various ages and physiological states. In 1998, the expert committee of the European Community in the Report on Osteoporosis-Action on prevention, has given the recommended daily dietary allowances (RDA) for calcium at all stage of life. For the elderly population, above age 65 the RDA is 700-800 mg/day. The main source of calcium in the diet are dairy products (milk, yoghurts and cheese) fish (sardines with bones), few vegetables and fruits. The optimal way to achieve adequate calcium intake is through the diet. However, when dietary sources are scarce or not well tolerated, calcium supplementation may be used. Calcium is generally well tolerated and reports of significant side-effects are rare. Adequate sunlight exposure may prevent and cure vitamin D insufficiency. However, the sunlight exposure or the ultraviolet irradiation are limited by concern about skin cancer and skin disease. The most rational approach to reducing vitamin D insufficiency is supplementation. In Europe, the RDA is 400-800 IU (10-20 microg) daily for people aged 65 years or over. This dose is safe and free of side effects. In conclusion, in Europe a low calcium intake and a suboptimal vitamin D status are very common in the elderly. Evidence supports routine supplementation for these people at risk of osteoporosis, by providing a daily intake of 700-800 mg of calcium and 400-800 IU of vitamin D. This is an effective, safe and cheap means of preventing osteoporotic fractures.

*Public Health Nutr.* 2001 Apr;4(2B):547-59

### **VITAMIN D DEFICIENCY IN HOMEBOUND ELDERLY PERSONS.**

**OBJECTIVE**—To assess the vitamin D status in homebound, community-dwelling elderly persons; sunlight-deprived elderly nursing home residents; and healthy, ambulatory elderly persons. **DESIGN**—A cohort analytic study. **PARTICIPANTS**—Of 244 subjects at least 65 years old, 116 subjects (85 women and 31 men) had been confined indoors for at least 6 months, either in private dwellings in the community (the Hopkins Elder Housecall Program) or in a teaching nursing home (The Johns Hopkins Geriatrics Center). The 128 control subjects, a healthy ambulatory group, came from the Baltimore Longitudinal Study on Aging. All subjects were free of diseases or medications that might interfere with their vitamin D status. **MAIN OUTCOME MEASURES**—Serum levels of 25-hydroxyvitamin D (25-OHD) and 1,25-dihydroxyvitamin D (1,25-[OH]2D) were measured in all subjects. In a subgroup of 80 subjects, serum levels of intact parathyroid hormone (PTH), ionized calcium, and osteocalcin and intake of vitamin D (through 3-day food records) were assessed. A randomly selected cohort of sunlight-deprived subjects also had serum levels of vitamin D binding protein measured. **RESULTS**—In sunlight-deprived subjects overall, the mean 25-OHD level was 30 nmol/L (12 ng/mL) (range, < 10 to 77 nmol/L [ $< 4$  to 31 ng/mL]) and the mean 1,25-(OH)2D level was 52 pmol/L (20 pg/mL) (range, 18 to 122 pmol/L [7 to 47 pg/mL]). In the sunlight-deprived subjects, 54% of community dwellers and 38% of nursing home residents had serum levels of 25-OHD below 25 nmol/L (10 ng/mL) (normal range, 25 to 137 nmol/L [10 to 55 ng/mL]). A significant inverse relationship existed between 25-OHD (ie, Log [25-OHD]) and PTH when they were analyzed together ( $r = -0.42$ ;  $R^2 = 0.18$ ;  $P < .001$ ) and for each cohort separately. All other parameters measured, except ionized calcium, differed significantly from the Baltimore Longitudinal Study Group means. The mean (SD) daily intakes of vitamin D (121 [132] IU) and calcium (583 [322] mg) were below the recommended dietary allowance only in the community-dwelling homebound population. The mean vitamin D binding protein level in the sunlight-deprived subgroup was in the normal range. **CONCLUSIONS**—Despite a relatively high degree of vitamin supplementation in the United States, homebound elderly persons are likely to suffer from vitamin D deficiency.

*JAMA.* 1995 Dec 6;274(21):1683-6

### **MAGNESIUM IN CLINICAL MEDICINE.**

Until recently the physiological role of magnesium was essentially ignored. However, with the development of new technologies to measure the intracellular free concentration of magnesium ( $[Mg^{2+}]_i$ ), the biologically important fraction, there has been an explosion of interest in the molecular, biochemical, physiological and pharmacological functions of magnesium. In addition improved methods for assessing magnesium status in the clinic have contributed to the further understanding of magnesium regulation in health and disease. Magnesium deficiency is now considered to contribute to many diseases and the role for magnesium as a therapeutic agent is being tested in numerous large clinical trials. This review focuses on clinical manifestations associated with magnesium deficiency and highlights the clinical significance of hypermagnesemia. Specific clinical conditions in which magnesium deficiency has been implicated to play a pathophysiological role, namely hypertension, ischemic heart disease, arrhythmias, pre-eclampsia, asthma and critical illness will be discussed and the possible therapeutic role of magnesium will be considered. Although there is still much to be learnt regarding the exact role of magnesium in clinical medicine, there are two conditions where magnesium is now considered the therapeutic agent of choice, pre-eclampsia and torsades de pointes. Future research, both at the fundamental and clinical levels, will certainly facilitate our understanding of how magnesium contributes to pathological processes and under what circumstances it should be used therapeutically.

*Front Biosci.* 2004 May 1;9:1278-93

### **MAGNESIUM STATUS AND AGEING: AN UPDATE.**

Ageing constitutes a risk factor for magnesium deficit. Primary magnesium deficit originates from two etiological mechanisms: deficiency and depletion. Primary magnesium deficiency is due to insufficient magnesium intake. Dietary amounts of magnesium are marginal in the whole population whatever the age. Nutritional deficiencies are more pronounced in institutionalized than in free-living ageing groups. Primary magnesium depletion is due to dysregulation of factors controlling magnesium status: intestinal magnesium hypoabsorption, reduced magnesium bone uptake and mobilisation, sometimes urinary leakage, hyperadrenoglucocorticism by decreased adaptability to stress, insulin-resistance and adrenergic hyporeceptivity. Secondary magnesium deficit in ageing largely results from various pathologies and treatments common to elderly persons: i.e. non insulin dependent diabetes mellitus and use of hypermagnesuric diuretics. Magnesium deficit may participate in the clinical pattern of ageing: mainly neuromuscular, cardiovascular and renal symptomatology. The consequences of hyperadrenoglucocorticism--whose non response to dexamethasone suppression test appears the simplest marker--may concern immunosuppression, muscle atrophy, centralization of fat mass, osteoporosis, hyperglycemia, hyperlipidemia, atherosclerosis, disturbances in mood and mental performances through accelerated hippocampal ageing particularly. Treatment of magnesium deficiency requires simple oral physiological magnesium supplementation. Treatment of the different types of magnesium depletion leads to a more or less specific control of pathophysiological disturbances of the required magnesium substrate. Open and double blind studies on the effects of the treatments of magnesium deficiency and of magnesium depletions in geriatric populations are too scarce. Further study is necessary to assess the accurate place of magnesium deficit in the physiopathology of ageing.

*Magnes Res.* 1998 Mar;11(1):25-42

### **INTRAVENOUS MAGNESIUM SULPHATE IN ACUTE MYOCARDIAL INFARCTION—IS THE ANSWER “MAGIC”?**

The role of magnesium in coronary artery disease has been evaluated extensively during the last three decades. The intravenous application of magnesium in acute myocardial infarction is of major importance, the beneficial effects have been underlined in several studies. Magnesium is of significance in the pathomechanisms of reperfusion injury and reduction of malignant arrhythmias in the critical acute phase of myocardial infarction, if applied intravenously. However, the promising results of LIMIT-2 could not be confirmed by the data of ISIS-4. The timing of magnesium therapy is probably the most important key factor. Similar to the guidelines of thrombolytic intervention, magnesium has to be administered as early as possible, at the latest before myocardial reperfusion has started. Nevertheless, because of conflicting results of prior trials doubts on the efficacy of intravenous magnesium in myocardial infarction still remain. The multinational, multicenter trial MAGIC has been set up to evaluate the optimal patient cohort as well as the ideal dose regimen for the application of intravenous magnesium sulphate in patients with acute myocardial infarction. The answer on the open questions on intravenous magnesium sulphate in myocardial infarction could be “MAGIC”.

## **CALCIUM:MAGNESIUM RATIO IN LOCAL GROUNDWATER AND INCIDENCE OF ACUTE MYOCARDIAL INFARCTION AMONG MALES IN RURAL FINLAND.**

Several epidemiologic studies have shown an association between calcium and magnesium and coronary heart disease mortality and morbidity. In this small-area study, we examined the relationship between acute myocardial infarction (AMI) risk and content of Ca, Mg, and chromium in local groundwater in Finnish rural areas using Bayesian modeling and geospatial data aggregated into 10 km times symbol 10 km grid cells. Data on 14,495 men 35-74 years of age with their first AMI in the years 1983, 1988, or 1993 were pooled. Geochemical data consisted of 4,300 measurements of each element in local groundwater. The median concentrations of Mg, Ca, and Cr and the Ca:Mg ratio in well water were 2.61 mg/L, 12.23 mg/L, 0.27 microg/L, and 5.39, respectively. Each 1 mg/L increment in Mg level decreased the AMI risk by 4.9%, whereas a one unit increment in the Ca:Mg ratio increased the risk by 3.1%. Ca and Cr did not show any statistically significant effect on the incidence and spatial variation of AMI. Results of this study with specific Bayesian statistical analysis support earlier findings of a protective role of Mg and low Ca:Mg ratio against coronary heart disease but do not support the earlier hypothesis of a protective role of Ca.

*Environ Health Perspect.* 2006 May;114(5):730-4

## **MAGNESIUM INTAKE AND INCIDENCE OF METABOLIC SYNDROME AMONG YOUNG ADULTS.**

**BACKGROUND:** Studies suggest that magnesium intake may be inversely related to risk of hypertension and type 2 diabetes mellitus and that higher intake of magnesium may decrease blood triglycerides and increase high-density lipoprotein (HDL) cholesterol levels. However, the longitudinal association of magnesium intake and incidence of metabolic syndrome has not been investigated. **METHODS AND RESULTS:** We prospectively examined the relations between magnesium intake and incident metabolic syndrome and its components among 4,637 Americans, aged 18 to 30 years, who were free from metabolic syndrome and diabetes at baseline. Metabolic syndrome was diagnosed according to the National Cholesterol Education Program/Adult Treatment Panel III definition. Diet was assessed by an interviewer-administered quantitative food frequency questionnaire, and magnesium intake was derived from the nutrient database developed by the Minnesota Nutrition Coordinating Center. During the 15 years of follow-up, 608 incident cases of the metabolic syndrome were identified. Magnesium intake was inversely associated with incidence of metabolic syndrome after adjustment for major lifestyle and dietary variables and baseline status of each component of the metabolic syndrome. Compared with those in the lowest quartile of magnesium intake, multivariable-adjusted hazard ratio of metabolic syndrome for participants in the highest quartile was 0.69 (95% confidence interval [CI], 0.52 to 0.91; P for trend <0.01). The inverse associations were not materially modified by gender and race. Magnesium intake was also inversely related to individual component of the metabolic syndrome and fasting insulin levels. **CONCLUSIONS:** Our findings suggest that young adults with higher magnesium intake have lower risk of development of metabolic syndrome.

*Circulation.* 2006 Apr 4;113(13):1675-82

## **CLINICAL EFFICACY OF MAGNESIUM SUPPLEMENTATION IN PATIENTS WITH TYPE 2 DIABETES.**

Effects of magnesium (Mg) supplementation on nine mild type 2 diabetic patients with stable glycemic control were investigated. Water from a salt lake with a high natural Mg content (7.1%) (MAG21) was used for supplementation after dilution with distilled water to 100mg/100mL; 300mL/day was given for 30 days. Fasting serum immunoreactive insulin level decreased significantly, as did HOMA squareR (both  $p < 0.05$ ). There was also a marked decrease of the mean triglyceride level after supplementation. The patients with hypertension showed significant reduction of systolic ( $p < 0.01$ ), diastolic ( $p = 0.0038$ ), and mean ( $p < 0.01$ ) blood pressure. The salt lake water supplement, MAG21, exerted clinical benefit as a Mg supplement in patients with mild type 2 diabetes mellitus.

*J Am Coll Nutr.* 2004 Oct;23(5):506S-509S

## **ROLE OF MAGNESIUM IN HYPERTENSION.**

Magnesium affects blood pressure by modulating vascular tone and reactivity. It acts as a calcium channel antagonist, it stimulates production of vasodilator prostacyclins and nitric oxide and it alters vascular responses to vasoactive agonists. Magnesium deficiency has been implicated in the pathogenesis of hypertension with epidemiological and experimental studies demonstrating an inverse correlation between blood pressure and serum magnesium levels. Magnesium also influences glucose and insulin homeostasis, and hypomagnesemia is associated with metabolic syndrome. Although most epidemiological and experimental studies support a role for low magnesium in the pathophysiology of hypertension, data from clinical studies have been less convincing. Furthermore, the therapeutic value of magnesium in the management of hypertension is unclear. The present review addresses the role of magnesium in the regulation of vascular function and blood pressure and discusses the implications of magnesium deficiency in experimental and clinical hypertension, in metabolic syndrome and in pre-eclampsia.

## **AEROSOLIZED MAGNESIUM SULFATE FOR ACUTE ASTHMA: A SYSTEMATIC REVIEW.**

**BACKGROUND:** The use of MgSO<sub>4</sub> is one of numerous treatment options available during exacerbations of asthma. While the efficacy of therapy with IV MgSO<sub>4</sub> has been demonstrated, little is known about inhaled MgSO<sub>4</sub>. **OBJECTIVES:** A systematic review of the literature was performed to examine the effect of inhaled MgSO<sub>4</sub> in the treatment of patients with asthma exacerbations in the emergency department. **METHODS:** Randomized controlled trials were eligible for inclusion and were identified from the Cochrane Airways Group "Asthma and Wheez\*" register, which consists of a combined search of the EMBASE, CENTRAL, MEDLINE, and CINAHL databases and the manual searching of 20 key respiratory journals. Reference lists of published studies were searched, and a review of the gray literature was also performed. Studies were included if patients had been treated with nebulized MgSO<sub>4</sub> alone or in combination with beta(2)-agonists and were compared to the use of beta(2)-agonists alone or with an inactive control substance. Trial selection, data extraction, and methodological quality were assessed by two independent reviewers. The results from fixed-effects models are presented as standardized mean differences (SMDs) for pulmonary functions and the relative risks (RRs) for hospital admission. Both are displayed with their 95% confidence intervals (CIs). **RESULTS:** Six trials involving 296 patients were included. There was a non-significant increase [corrected] in pulmonary function between patients whose treatments included nebulized MgSO<sub>4</sub> and those whose treatments [corrected] did not (SMD, 0.22; 95% CI, -0.02 to 0.47 [corrected] five studies); there was also a trend toward reduced [corrected] hospitalizations in patients whose treatments included nebulized MgSO<sub>4</sub> (RR, 0.67; 95% CI, 0.41 to 1.09; four studies). Subgroup analyses demonstrated that lung function improvement was similar in adult patients and in those patients who received nebulized MgSO<sub>4</sub> in addition to a beta(2)-agonist. **CONCLUSIONS:** The use of nebulized MgSO<sub>4</sub>, particularly in addition to a beta(2)-agonist, in the treatment of an acute asthma exacerbation appears to produce benefits with respect to improved pulmonary function and may reduce the number of hospital admissions.

*Chest. 2005 Jul;128(1):337-44*

## **MAGNESIUM DEFICIENCY AND OSTEOPOROSIS: ANIMAL AND HUMAN OBSERVATIONS.**

Although osteoporosis is a major health concern for our growing population of the elderly, there continues to be a need for well-designed clinical and animal studies on the link between dietary magnesium (Mg) intake and osteoporosis. Relatively few animal studies have assessed the skeletal and hormonal impact of long-term low Mg intake; however, these studies have demonstrated that Mg deficiency results in bone loss. Potential mechanisms include a substance P-induced release of inflammatory cytokines as well as impaired production of parathyroid hormone and 1,25-dihydroxyvitamin D. Abnormal mineralization of bones may also contribute to skeletal fragility. Clinical studies have often varied greatly in study design, subject age, menopausal status and outcome variables that were assessed. Most studies focused on female subjects, thus pointing to the great need for studies on aging males. According to the U.S. Department of Agriculture, the mean Mg intake for males and females is 323 and 228 mg/day, respectively. These intake levels suggest that a substantial number of people may be at risk for Mg deficiency, especially if concomitant disorders and/or medications place the individual at further risk for Mg depletion. In this paper, we will review animal and human evidence of the association of Mg deficiency with osteoporosis and explore possible mechanisms by which this may occur.

*J Nutr Biochem. 2004 Dec;15(12):710-6*

## **BIOAVAILABILITY OF US COMMERCIAL MAGNESIUM PREPARATIONS.**

Magnesium deficiency is seen with some frequency in the outpatient setting and requires oral repletion or maintenance therapy. The purpose of this study was to measure the bioavailability of four commercially-available preparations of magnesium, and to test the claim that organic salts are more easily absorbed. Bioavailability was measured as the increment of urinary magnesium excretion in normal volunteers given approximately 21 mEq/day of the test preparations. Results indicated relatively poor bioavailability of magnesium oxide (fractional absorption 4 per cent) but significantly higher and equivalent bioavailability of magnesium chloride, magnesium lactate and magnesium aspartate. We conclude that there is relatively poor bioavailability of magnesium oxide, but greater and equivalent bioavailability of magnesium chloride, lactate, and aspartate. Inorganic magnesium salts, depending on the preparation, may have bioavailability equivalent to organic magnesium salts.

*Magnes Res. 2001 Dec;14(4):257-62*

## **STUDY OF MAGNESIUM BIOAVAILABILITY FROM TEN ORGANIC AND INORGANIC MG SALTS IN MG-DEPLETED RATS USING A STABLE ISOTOPE APPROACH.**

Literature data on the bioavailability of various Mg forms provide scarce information on the best Mg salt to be used in animal and human supplementation. This study aimed to investigate the bioavailability of different forms of Mg in rats using Mg stable isotopes. Eighty male Wistar rats aged 6 weeks were fed a semi-purified Mg-depleted diet for three weeks. The rats were then

randomised into ten groups and received, for two more weeks, the same diet repleted with Mg (550 mg Mg/kg) as: oxide, chloride, sulphate, carbonate, acetate, pidolate, citrate, gluconate, lactate or aspartate. After 10 days of Mg-repleted diet, the rats received orally 1.8 mg of an enriched  $^{26}\text{Mg}$ . Faeces and urine were then collected for 4 consecutive days. Isotope ratios in faeces and urine were determined. The Mg absorption values obtained varied from 50% to 67%. Organic Mg salts were slightly more available than inorganic Mg salts. Mg gluconate exhibited the highest Mg bioavailability of the ten Mg salts studied. Urinary  $^{26}\text{Mg}$  excretion varied from 0.20 mg to 0.33 mg, and feeding with the organic pidolate, citrate, gluconate and aspartate salts resulted in higher urinary  $^{26}\text{Mg}$  excretion than with inorganic salts. Ultimately,  $^{26}\text{Mg}$  retention was higher in the rats receiving the organic salts such as gluconate, lactate and aspartate than in those receiving the inorganic salts. Taken together, these results indicate that  $^{26}\text{Mg}$  is sufficiently bioavailable from the ten different Mg salts studied in the present experiment, although Mg gluconate exhibited the highest bioavailability under these experimental conditions.

*Magnes Res. 2005 Dec;18(4):215-23*

## ORAL URIDINE-5'-MONOPHOSPHATE (UMP) INCREASES BRAIN CDP-CHOLINE LEVELS IN GERBILS.

We examined the biochemical pathways whereby oral uridine-5'-monophosphate (UMP) increases membrane phosphatide synthesis in brains of gerbils. We previously showed that supplementing PC12 cells with uridine caused concentration-related increases in CDP-choline levels, and that this effect was mediated by elevations in intracellular uridine triphosphate (UTP) and cytidine triphosphate (CTP). In the present study, adult gerbils received UMP (1 mmol/kg), a constituent of human breast milk and infant formulas, by gavage, and plasma samples and brains were collected for assay between 5 min and 8 h thereafter. Thirty minutes after gavage, plasma uridine levels were increased from 6.6 +/- 0.58 to 32.7 +/- 1.85  $\mu$ M ( $P < 0.001$ ), and brain uridine from 22.6 +/- 2.9 to 89.1 +/- 8.82 pmol/mg tissue ( $P < 0.001$ ). UMP also significantly increased plasma and brain cytidine levels; however, both basally and following UMP, these levels were much lower than those of uridine. Brain UTP, CTP, and CDP-choline were all elevated 15 min after UMP (from 254 +/- 31.9 to 417 +/- 50.2, [ $P < 0.05$ ]; 56.8 +/- 1.8 to 71.7 +/- 1.8, [ $P < 0.001$ ]; and 11.3 +/- 0.5 to 16.4 +/- 1, [ $P < 0.001$ ] pmol/mg tissue, respectively), returning to basal levels after 20 and 30 min. The smallest UMP dose that significantly increased brain CDP-choline was 0.05 mmol/kg. These results show that oral UMP, a uridine source, enhances the synthesis of CDP-choline, the immediate precursor of PC, in gerbil brain.

*Brain Res. 2005 Oct 5;1058(1-2):101-8*

## SYNAPTIC PROTEINS AND PHOSPHOLIPIDS ARE INCREASED IN GERBIL BRAIN BY ADMINISTERING URIDINE PLUS DOCOSAHEXAENOIC ACID ORALLY.

The synthesis of brain phosphatidylcholine may utilize three circulating precursors: choline; a pyrimidine (e.g., uridine, converted via UTP to brain CTP); and a PUFA (e.g., docosahexaenoic acid); phosphatidylethanolamine may utilize two of these, a pyrimidine and a PUFA. We observe that consuming these precursors can substantially increase membrane phosphatide and synaptic protein levels in gerbil brains. (Pyrimidine metabolism in gerbils, but not rats, resembles that in humans.) Animals received, daily for 4 weeks, a diet containing choline chloride and UMP (a uridine source) and/or DHA by gavage. Brain phosphatidylcholine rose by 13-22% with uridine and choline alone, or DHA alone, or by 45% with the combination, phosphatidylethanolamine and the other phosphatides increasing by 39-74%. Smaller elevations occurred after 1-3 weeks. The combination also increased the vesicular protein Synapsin-1 by 41%, the postsynaptic protein PSD-95 by 38% and the neurite neurofibrillar proteins NF-70 and NF-M by up to 102% and 48%, respectively. However, it had no effect on the cytoskeletal protein beta-tubulin. Hence, the quantity of synaptic membrane probably increased. The precursors act by enhancing the substrate saturation of enzymes that initiate their incorporation into phosphatidylcholine and phosphatidylethanolamine and by UTP-mediated activation of P2Y receptors. Alzheimer's disease brains contain fewer and smaller synapses and reduced levels of synaptic proteins, membrane phosphatides, choline and DHA. The three phosphatide precursors might thus be useful in treating this disease.

*Brain Res. 2006 May 9;1088(1):83-92*

## DIETARY URIDINE-5'-MONOPHOSPHATE SUPPLEMENTATION INCREASES POTASSIUM-EVOKED DOPAMINE RELEASE AND PROMOTES NEURITE OUTGROWTH IN AGED RATS.

Membrane phospholipids like phosphatidylcholine (PC) are required for cellular growth and repair, and specifically for synaptic function. PC synthesis is controlled by cellular levels of its precursor, cytidine-5'-diphosphate choline (CDP-choline), which is produced from cytidine triphosphate (CTP) and phosphocholine. In rat PC12 cells exogenous uridine was shown to elevate intracellular CDP-choline levels, by promoting the synthesis of uridine triphosphate (UTP), which was partly converted to CTP. In such cells uridine also enhanced the neurite outgrowth produced by nerve growth factor (NGF). The present study assessed the effect of dietary supplementation with uridine-5'-monophosphate disodium (UMP-2Na<sup>+</sup>, an additive in infant milk formulas) on striatal dopamine (DA) release in aged rats. Male Fischer 344 rats consumed either a control diet or one fortified with 2.5% UMP for 6 wk, ad libitum. In vivo microdialysis was then used to measure spontaneous and potassium (K<sup>+</sup>)-evoked DA release in the right striatum. Potassium (K<sup>+</sup>)-evoked DA release was significantly greater among UMP-treated rats, i.e., 341 +/- 21% of basal levels vs. 283 +/- 9% of basal levels in control rats ( $p < 0.05$ ); basal DA release was unchanged. In general, each animal's K<sup>+</sup>-evoked DA release correlated with its striatal DA content, measured postmortem. The levels of neurofilament-70 and neurofilament-M proteins, biomarkers of neurite outgrowth, increased to 182 +/- 25% ( $p < 0.05$ ) and 221 +/- 34% ( $p < 0.01$ ) of control values, respectively, with UMP consumption. Hence, UMP treatment not only enhances membrane phosphatide production but

also can modulate two membrane-dependent processes, neurotransmitter release and neurite outgrowth, in vivo.

*J Mol Neurosci.* 2005;27(1):137-45

### **URIDINE ENHANCES NEURITE OUTGROWTH IN NERVE GROWTH FACTOR-DIFFERENTIATED PC12 [CORRECTED]**

During rapid cell growth the availability of phospholipid precursors like cytidine triphosphate and diacylglycerol can become limiting in the formation of key membrane constituents like phosphatidylcholine. Uridine, a normal plasma constituent, can be converted to cytidine triphosphate in PC12 [corrected] cells and intact brain, and has been shown to produce a resulting increase in phosphatidylcholine synthesis. To determine whether treatments that elevate uridine availability also thereby augment membrane production, we exposed PC12 [corrected] cells which had been differentiated by nerve growth factor to various concentrations of uridine, and measured the numbers of neurites the cells produced. After 4 but not 2 days uridine significantly and dose-dependently increased the number of neurites per cell. This increase was accompanied by increases in neurite branching and in levels of the neurite proteins neurofilament M [corrected] and neurofilament 70. Uridine treatment also increased intracellular levels of cytidine triphosphate, which suggests that uridine may affect neurite outgrowth by enhancing phosphatidylcholine synthesis. Uridine may also stimulate neuritogenesis by a second mechanism, since the increase in neurite outgrowth was mimicked by exposing the cells to uridine triphosphate, and could be blocked by various drugs known to antagonize P2Y receptors (suramin; Reactive Blue 2; pyridoxal-phosphate-6-azophenyl-2',4' disulfonic acid). Treatment of the cells with uridine or uridine triphosphate stimulated their accumulation of inositol phosphates, and this effect was also blocked by pyridoxal-phosphate-6-azophenyl-2',4' disulfonic acid. Moreover, degradation of nucleotides by apyrase blocked the stimulatory effect of uridine on neuritogenesis. Taken together these data indicate that uridine can regulate the output of neurites from differentiating PC12 [corrected] cells, and suggest that it does so in two ways, i.e. both by acting through cytidine triphosphate as a precursor for phosphatidylcholine biosynthesis and through uridine triphosphate as an agonist for P2Y receptors.

*Neuroscience.* 2005;134(1):207-14

### **CHRONIC ADMINISTRATION OF UMP AMELIORATES THE IMPAIRMENT OF HIPPOCAMPAL-DEPENDENT MEMORY IN IMPOVERISHED RATS.**

We have previously shown that chronic, but not acute, dietary supplementation with CDP-choline prevents the hippocampal-dependent memory deficits manifested by aged rats and by rats reared under impoverished environmental conditions. In rats, dietary CDP-choline is rapidly metabolized into cytidine and choline; the cytidine is then readily converted to uridine, which enters the brain and, via conversion to UTP and CTP, increases brain levels of membrane phosphatides. Hence, we have assessed whether administering a uridine source (UMP) instead of CDP-choline can also ameliorate the memory deficits in rats reared under impoverished environmental conditions. At weaning, 32 male Sprague-Dawley rats were exposed to either enriched (EC) or impoverished (IC) conditions for 3 mo. Concurrently, IC and EC rats were given access to either a control diet or a diet supplemented with 0.1% UMP. Rats were then assessed for learning and memory skills using 2 versions of the Morris water maze, the hidden platform version that assesses hippocampal-dependent cognitive memory processing, and the visible platform version that assesses striatal-dependent habit memory. As expected, exposure to the impoverished environment impaired hippocampal-dependent, but not striatal-dependent learning and memory. Supplementation with UMP prevented this cognitive dysfunction, as had been observed with supplemental CDP-choline. These results suggest that IC rats do not use and/or remember their spatial strategies for task solving as well as EC rats, and that long-term dietary supplementation with UMP alleviates this dysfunction.

*J Nutr.* 2006 Nov;136(11):2834-7

### **NEURODEGENERATION FROM MITOCHONDRIAL INSUFFICIENCY: NUTRIENTS, STEM CELLS, GROWTH FACTORS, AND PROSPECTS FOR BRAIN REBUILDING USING INTEGRATIVE MANAGEMENT.**

Degenerative brain disorders (neurodegeneration) can be frustrating for both conventional and alternative practitioners. A more comprehensive, integrative approach is urgently needed. One emerging focus for intervention is brain energetics. Specifically, mitochondrial insufficiency contributes to the etiopathology of many such disorders. Electron leakages inherent to mitochondrial energetics generate reactive oxygen free radical species that may place the ultimate limit on lifespan. Exogenous toxins, such as mercury and other environmental contaminants, exacerbate mitochondrial electron leakage, hastening their demise and that of their host cells. Studies of the brain in Alzheimer's and other dementias, Down syndrome, stroke, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, Huntington's disease, Friedreich's ataxia, aging, and constitutive disorders demonstrate impairments of the mitochondrial citric acid cycle and oxidative phosphorylation (OXPHOS) enzymes. Imaging or metabolic assays frequently reveal energetic insufficiency and depleted energy reserve in brain tissue in situ. Orthomolecular nutrients involved in mitochondrial metabolism provide clinical benefit. Among these are the essential minerals and the B vitamin group; vitamins E and K; and the antioxidant and energetic cofactors alpha-lipoic acid (ALA), ubiquinone (coenzyme Q10; CoQ10), and nicotinamide adenine dinucleotide, reduced (NADH). Recent advances in the area of stem cells and growth factors encourage

optimism regarding brain regeneration. The protein nutrients acetyl L-carnitine (ALCAR), glycerophosphocholine (GPC), and phosphatidylserine (PS) provide mitochondrial support and conserve growth factor receptors; all three improved cognition in double-blind trials. The omega-3 fatty acid docosahexaenoic acid (DHA) is enzymatically combined with GPC and PS to form membrane phospholipids for nerve cell expansion. Practical recommendations are presented for integrating these safe and well-tolerated orthomolecular nutrients into a comprehensive dietary supplementation program for brain vitality and productive life span.

*Altern Med Rev. 2005 Dec;10(4):268-93*

### **NEUROPROTECTIVE EFFECTS OF WITHANIA SOMNIFERA ON 6-HYDROXYDOPAMINE INDUCED PARKINSONISM IN RATS.**

6-Hydroxydopamine (6-OHDA) is one of the most widely used rat models for Parkinson's disease. There is ample evidence in the literature that 6-OHDA elicits its toxic manifestations through oxidant stress. In the present study, we evaluated the anti-parkinsonian effects of *Withania somnifera* extract, which has been reported to have potent anti-oxidant, anti-peroxidative and free radical quenching properties in various diseased conditions. Rats were pretreated with 100, 200 and 300 mg/kg b.w. of the *W. somnifera* extract orally for 3 weeks. On day 21, 2 microL of 6-OHDA (10 microg in 0.1% in ascorbic acid-saline) was infused into the right striatum while sham operated group received 2 microL of the vehicle. Three weeks after 6-OHDA injections, rats were tested for neurobehavioral activity and were killed 5 weeks after lesioning for the estimation of lipidperoxidation, reduced glutathione content, activities of glutathione-S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase, catecholamine content, dopaminergic D2 receptor binding and tyrosine hydroxylase expression. *W. somnifera* extract was found to reverse all the parameters significantly in a dose-dependent manner. Thus, the study demonstrates that the extract of *W. somnifera* may be helpful in protecting the neuronal injury in Parkinson's disease.

*Hum Exp Toxicol. 2005 Mar;24(3):137-47*

### **GRAPE SEED PROANTHOCYANIDIN EXTRACT (GSPE) AND ANTIOXIDANT DEFENSE IN THE BRAIN OF ADULT RATS.**

**BACKGROUND:** Proanthocyanidin (PA) is a naturally occurring antioxidant from grape seed extract. The present study aims at assessing the neuroprotective effects of grape seed proanthocyanidin (GSPE) on the cerebral cortex (CC), cerebellum (CB), and hippocampus (HC) in the adult rat brain. **MATERIAL/METHODS:** GSPE was orally administered at 25, 50, and 75 mg per kg body weight daily and for a total period of 9 weeks. Antioxidant enzymes (AOEs), superoxide dismutase (SOD), and catalase (CAT) were analyzed along with malondialdehyde (MDA) and protein carbonyl content (PCC) as markers of lipid peroxidation (LPO) and protein oxidation (PO). The cholinergic system was studied by analyzing choline acetyl transferase (ChAT) and acetylcholine esterase (AChE) activities along with acetylcholine content (ACh). **RESULTS:** The results obtained revealed an increased SOD activity in the 75-mg PA-supplemented animals, with a substantial decrease in MDA and PCC. The cholinergic neurotransmitter system analysis showed increased ChAT activity indicative of increased ACh content in the supplemented animals and the increase was more in the 75-mg PA group with a concomitant and moderate decrease in AChE activity. Regional changes were more with reference to HC. **CONCLUSIONS:** Our study shows that PA intake in moderately low quantity is effective in up-regulating the antioxidant defense mechanism by attenuating LPO and PO. Changes in the cholinergic system, however, indicate an increase in the ACh concentration with a moderate reduction in AChE activity, suggesting further that PA may have a potent role in enhancing cognition in older rats.

*Med Sci Monit. 2006 Apr;12(4):BR124-9*

### **BLUEBERRY SUPPLEMENTED DIET REVERSES AGE-RELATED DECLINE IN HIPPOCAMPAL HSP70 NEUROPROTECTION.**

Dietary supplementation with antioxidant rich foods can decrease the level of oxidative stress in brain regions and can ameliorate age-related deficits in neuronal and behavioral functions. We examined whether short-term supplementation with blueberries might enhance the brain's ability to generate a heat shock protein 70 (HSP70) mediated neuroprotective response to stress. Hippocampal (HC) regions from young and old rats fed either a control or a supplemented diet for 10 weeks were subjected to an in vitro inflammatory challenge (LPS) and then examined for levels of HSP70 at various times post LPS (30, 90 and 240 min). While baseline levels of HSP70 did not differ among the various groups compared to young control diet rats, increases in HSP70 protein levels in response to an in vitro LPS challenge were significantly less in old as compared to young control diet rats at the 30, 90, and 240 min time points. However, it appeared that the blueberry diet completely restored the HSP70 response to LPS in the old rats at the 90 and 240 min times. This suggests that a short-term blueberry (BB) intervention may result in improved HSP70-mediated protection against a number of neurodegenerative processes in the brain. Results are discussed in terms of the multiplicity of the effects of the BB supplementation which appear to range from antioxidant/anti-inflammatory activity to signaling.

**MODULATORY ROLE OF GRAPE SEED EXTRACT ON AGE-RELATED OXIDATIVE DNA DAMAGE IN CENTRAL NERVOUS SYSTEM OF RATS.**

Aging is the accumulation of diverse deleterious changes in the cells and tissues leading to increased risk of diseases. Oxidative stress is considered as a major risk factor and contributes to age related increase in DNA oxidation and DNA protein cross-links in central nervous system during aging. In the present study, we have evaluated the salubrious role of grape seed extract on accumulation of oxidative DNA damage products such as 8-OHdG and DNA protein cross-links in aged rats. Male albino rats of Wistar strain were divided into four groups: Group I, young control rats; Group II, young rats treated with grape seed extract (100mg/kgb.wt.) for 30 days; Group III, aged control rats; Group IV, aged rats supplemented with grape seed extract (100mg/kgb.wt.) for 30 days. Our results, thus, revealed that grape seed extract has inhibiting effect on the accumulation of age-related oxidative DNA damages in spinal cord and in various brain regions such as cerebral cortex, striatum and hippocampus.

*Brain Res Bull. 2006 Feb 15;68(6):469-73*

**PHASE II STUDY OF POMEGRANATE JUICE FOR MEN WITH RISING PROSTATE-SPECIFIC ANTIGEN FOLLOWING SURGERY OR RADIATION FOR PROSTATE CANCER.**

**PURPOSE:** Phytochemicals in plants may have cancer preventive benefits through antioxidation and via gene-nutrient interactions. We sought to determine the effects of pomegranate juice (a major source of antioxidants) consumption on prostate-specific antigen (PSA) progression in men with a rising PSA following primary therapy. **EXPERIMENTAL DESIGN:** A phase II, Simon two-stage clinical trial for men with rising PSA after surgery or radiotherapy was conducted. Eligible patients had a detectable PSA > 0.2 and < 5 ng/mL and Gleason score < or = 7. Patients were treated with 8 ounces of pomegranate juice daily (Wonderful variety, 570 mg total polyphenol gallic acid equivalents) until disease progression. Clinical end points included safety and effect on serum PSA, serum-induced proliferation and apoptosis of LNCaP cells, serum lipid peroxidation, and serum nitric oxide levels. **RESULTS:** The study was fully accrued after efficacy criteria were met. There were no serious adverse events reported and the treatment was well tolerated. Mean PSA doubling time significantly increased with treatment from a mean of 15 months at baseline to 54 months posttreatment ( $P < 0.001$ ). In vitro assays comparing pretreatment and posttreatment patient serum on the growth of LNCaP showed a 12% decrease in cell proliferation and a 17% increase in apoptosis ( $P = 0.0048$  and  $0.0004$ , respectively), a 23% increase in serum nitric oxide ( $P = 0.0085$ ), and significant ( $P < 0.02$ ) reductions in oxidative state and sensitivity to oxidation of serum lipids after versus before pomegranate juice consumption. **CONCLUSIONS:** We report the first clinical trial of pomegranate juice in patients with prostate cancer. The statistically significant prolongation of PSA doubling time, coupled with corresponding laboratory effects on prostate cancer in vitro cell proliferation and apoptosis as well as oxidative stress, warrant further testing in a placebo-controlled study.

*Clin Cancer Res. 2006 Jul 1;12(13):4018-26*

**POMEGRANATE FLOWER IMPROVES CARDIAC LIPID METABOLISM IN A DIABETIC RAT MODEL: ROLE OF LOWERING CIRCULATING LIPIDS.**

Excess triglyceride (TG) accumulation and increased fatty acid (FA) oxidation in the diabetic heart contribute to cardiac dysfunction. Punica granatum flower (PGF) is a traditional antidiabetic medicine. Here, we investigated the effects and mechanisms of action of PGF extract on abnormal cardiac lipid metabolism both in vivo and in vitro. Long-term oral administration of PGF extract (500 mg kg<sup>-1</sup>) reduced cardiac TG content, accompanied by a decrease in plasma levels of TG and total cholesterol in Zucker diabetic fatty (ZDF) rats, indicating improvement by PGF extract of abnormal cardiac TG accumulation and hyperlipidemia in this diabetic model. Treatment of ZDF rats with PGF extract lowered plasma FA levels. Furthermore, the treatment suppressed cardiac overexpression of mRNAs encoding for FA transport protein, peroxisome proliferator-activated receptor (PPAR)-alpha, carnitine palmitoyltransferase-1, acyl-CoA oxidase and 5'-AMP-activated protein kinase alpha2, and restored downregulated cardiac acetyl-CoA carboxylase mRNA expression in ZDF rats, whereas it showed little effect in Zucker lean rats. The results suggest that PGF extract inhibits increased cardiac FA uptake and oxidation in the diabetic condition. PGF extract and its component oleanolic acid enhanced PPAR-alpha luciferase reporter gene activity in human embryonic kidney 293 cells, and this effect was completely suppressed by a selective PPAR-alpha antagonist MK-886, consistent with the presence of PPAR-alpha activator activity in the extract and this component. Our findings suggest that PGF extract improves abnormal cardiac lipid metabolism in ZDF rats by activating PPAR-alpha and thereby lowering circulating lipid and inhibiting its cardiac uptake.

*Br J Pharmacol. 2005 Jul;145(6):767-74*

**POMEGRANATE JUICE INHIBITS OXIDIZED LDL UPTAKE AND CHOLESTEROL BIOSYNTHESIS IN MACROPHAGES.**

Macrophage cholesterol accumulation and foam cell formation are the hallmarks of early atherogenesis. Pomegranate juice (PJ) was shown to inhibit macrophage foam cell formation and development of atherosclerotic lesions. The aim of this study was to elucidate possible mechanisms by which PJ reduces cholesterol accumulation in macrophages. J774.A1 macrophages were preincubated with PJ followed by analysis of cholesterol influx [evaluated as LDL or as oxidized LDL (Ox-LDL) cellular degradation], cholesterol efflux and cholesterol biosynthesis. Preincubation of macrophages with PJ resulted in a significant reduction ( $P < .01$ ) in Ox-LDL degradation by 40%. On the contrary, PJ had no effect on macrophage degradation of native LDL or on macrophage cholesterol efflux. Macrophage cholesterol biosynthesis was inhibited by 50% ( $P < .01$ ) after cell incubation with

PJ. This inhibition, however, was not mediated at the 3-hydroxy-3-methylglutaryl coenzyme A reductase level along the biosynthetic pathway. We conclude that PJ-mediated suppression of Ox-LDL degradation and of cholesterol biosynthesis in macrophages can lead to reduced cellular cholesterol accumulation and foam cell formation.

*J Nutr Biochem.* 2005 Sep;16(9):570-6

### **POMEGRANATE JUICE PROTECTS NITRIC OXIDE AGAINST OXIDATIVE DESTRUCTION AND ENHANCES THE BIOLOGICAL ACTIONS OF NITRIC OXIDE.**

Pomegranate juice (PJ), which is a rich source of potent flavonoid antioxidants, was tested for its capacity to protect nitric oxide (NO) against oxidative destruction and enhance the biological actions of NO. Employing chemiluminescence headspace analysis, PJ was found to be a potent inhibitor of superoxide anion-mediated disappearance of NO. PJ was much more potent than Concord grape juice, blueberry juice, red wine, ascorbic acid, and DL-alpha-tocopherol. As little as 3 microl of a 6-fold dilution of PJ, in a reaction volume of 5000 microl, produced a marked antioxidant effect, whereas 300 microl of undiluted blueberry juice or nearly 1000 microl of undiluted Concord grape juice were required to produce similar effects. PJ and other antioxidant-containing products were found to augment the anti-proliferative action of NO (DETA/NO) on vascular smooth muscle cell (rat aorta) proliferation. PJ was much more effective than the other products tested and elicited no effects when tested alone in the absence of added NO. Similarly, neither PJ nor the other products enhanced the anti-proliferative action of alpha-difluoromethylornithine, a stable substance that inhibits cell growth by NO-independent mechanisms. In order to determine whether PJ is capable of increasing the production of NO by vascular endothelial cells, PJ was tested for its capacity to upregulate and/or activate endothelial NO synthase (eNOS) in bovine pulmonary artery endothelial cells. PJ elicited no effects on eNOS protein expression or catalytic activity. Moreover, PJ did not enhance promoter activity in the eNOS gene (COS-7 cells transfected with eNOS). These observations indicate that PJ possesses potent antioxidant activity that results in marked protection of NO against oxidative destruction, thereby resulting in augmentation of the biological actions of NO.

*Nitric Oxide.* 2006 Sep;15(2):93-102

### **PROSTATE CANCER PREVENTION THROUGH POMEGRANATE FRUIT.**

Prostate cancer (CaP) is the second leading cause of cancer-related deaths among U.S. males with a similar trend in many Western countries. CaP is an ideal candidate disease for chemoprevention because it is typically diagnosed in men over 50 years of age, and thus even a modest delay in disease progression achieved through pharmacological or nutritional intervention could significantly impact the quality of life of these patients. In this regard we and others have proposed the use of dietary antioxidants as candidate CaP chemopreventive agents. The fruit pomegranate derived from the tree *Punica granatum* has been shown to possess strong antioxidant and anti-inflammatory properties. In a recent study, we showed that pomegranate fruit extract (PFE), through modulations in the cyclin kinase inhibitor-cyclin-dependent kinase machinery, resulted in inhibition of cell growth followed by apoptosis of highly aggressive human prostate carcinoma PC3 cells. These events were associated with alterations in the levels of Bax and Bcl-2 shifting the Bax:Bcl-2 ratio in favor of apoptosis. Further, we showed that oral administration of a human acceptable dose of PFE to athymic nude mice implanted with CWR22Rnu1 cells resulted in significant inhibition of tumor growth with concomitant reduction in secretion of prostate-specific antigen (PSA) in the serum. The outcome of this study could have a direct practical implication and translational relevance to CaP patients, because it suggests that pomegranate consumption may retard CaP progression, which may prolong the survival and quality of life of the patients.

*Cell Cycle.* 2006 Feb;5(4):371-3

### **POMEGRANATE FRUIT JUICE FOR CHEMOPREVENTION AND CHEMOTHERAPY OF PROSTATE CANCER.**

Prostate cancer is the most common invasive malignancy and the second leading cause of cancer-related deaths among U.S. males, with a similar trend in many Western countries. One approach to control this malignancy is its prevention through the use of agents present in diet consumed by humans. Pomegranate from the tree *Punica granatum* possesses strong antioxidant and antiinflammatory properties. We recently showed that pomegranate fruit extract (PFE) possesses remarkable antitumor-promoting effects in mouse skin. In this study, employing human prostate cancer cells, we evaluated the antiproliferative and proapoptotic properties of PFE. PFE (10-100 microg/ml; 48 h) treatment of highly aggressive human prostate cancer PC3 cells resulted in a dose-dependent inhibition of cell growth/cell viability and induction of apoptosis. Immunoblot analysis revealed that PFE treatment of PC3 cells resulted in (i) induction of Bax and Bak (proapoptotic); (ii) down-regulation of Bcl-X(L) and Bcl-2 (antiapoptotic); (iii) induction of WAF1/p21 and KIP1/p27; (iv) a decrease in cyclins D1, D2, and E; and (v) a decrease in cyclin-dependent kinase (cdk) 2, cdk4, and cdk6 expression. These data establish the involvement of the cyclin kinase inhibitor-cyclin-cdk network during the antiproliferative effects of PFE. Oral administration of PFE (0.1% and 0.2%, wt/vol) to athymic nude mice implanted with androgen-sensitive CWR22Rnu1 cells resulted in a significant inhibition in tumor growth concomitant with a significant decrease in serum prostate-specific antigen levels. We suggest that pomegranate juice may have cancer-chemopreventive as well as cancer-chemotherapeutic effects against prostate cancer in humans.

### **POMEGRANATE JUICE REDUCES OXIDIZED LOW-DENSITY LIPOPROTEIN DOWNREGULATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASE IN HUMAN CORONARY ENDOTHELIAL CELLS.**

We examined the hypothesis that pomegranate juice (PJ) can revert the potent downregulation of the expression of endothelial nitric-oxide synthase (NOSIII) induced by oxidized low-density lipoprotein (oxLDL) in human coronary endothelial cells. Western blot and Northern blot analyses showed a significant decrease of NOSIII expression after a 24-h treatment with oxLDL. Accordingly, we observed a significant dose-dependent reduction in nitric oxide bioactivity represented by both basal and bradykinin-stimulated cellular cGMP accumulation. These phenomena were corrected significantly by the concomitant treatment with PJ. Our data suggest that PJ can exert beneficial effects on the evolution of clinical vascular complications, coronary heart disease, and atherogenesis in humans by enhancing the NOSIII bioactivity.

*Nitric Oxide. 2006 Nov;15(3):259-63*

### **POMEGRANATE BYPRODUCT ADMINISTRATION TO APOLIPOPROTEIN E-DEFICIENT MICE ATTENUATES ATHEROSCLEROSIS DEVELOPMENT AS A RESULT OF DECREASED MACROPHAGE OXIDATIVE STRESS AND REDUCED CELLULAR UPTAKE OF OXIDIZED LOW-DENSITY LIPOPROTEIN.**

The effects of a pomegranate byproduct (PBP, which includes the whole pomegranate fruit left after juice preparation) on atherosclerosis development in apolipoprotein E-deficient (E degrees) mice were studied. Consumption of PBP (17 or 51.5 microg of gallic acid equiv/kg/day) by the mice resulted in a significant reduction in atherosclerotic lesion size by up to 57%. PBP consumption significantly reduced oxidative stress in the mice peritoneal macrophages (MPM): Cellular lipid peroxide content decreased by up to 42%, the reduced glutathione levels increased by up to 53%, and paraoxonase 2 lactonase activity increased by up to 50%, as compared to MPM from E degrees mice that consumed only water. Furthermore, oxidized low-density lipoprotein (Ox-LDL) uptake by the MPM was reduced by up to 19%. Similar results were observed also in vitro. Treatment of J774A.1 macrophages with PBP (10 or 50 micromol/L of total polyphenols) significantly decreased both cellular total peroxide content and Ox-LDL uptake. It was thus concluded that PBP significantly attenuates atherosclerosis development by its antioxidant properties.

*J Agric Food Chem. 2006 Mar 8;54(5):1928-35*

### **A RETROSPECTIVE STUDY OF THE RELATIONSHIP BETWEEN BIOMARKERS OF ATHEROSCLEROSIS AND ERECTILE DYSFUNCTION IN 988 MEN.**

Erectile dysfunction (ED) is associated with clinical atherosclerosis and several atherosclerotic risk factors including smoking, hypertension, dyslipidemia, diabetes mellitus, obesity and sedentary lifestyle. Clinical atherosclerosis is also associated with these same risk factors and with biomarkers of inflammation, thrombosis, endothelial cell activation. We evaluated the cross-sectional association between the degree of ED and levels of atherosclerotic biomarkers. A subcohort of 988 US male health professionals between the ages 46 and 81 years as part of an ongoing epidemiologic study had atherosclerotic biomarkers measured from blood collected in 1994-1995. These same men had in 2000, been retrospectively asked about erectile function in 1995 and in 2000. Biennial questionnaires since 1986 assessed medical conditions, medications, smoking, physical activity, body mass index, alcohol intake. The retrospective assessment of erectile function in 2000 for 1995 in these 988 men ranged from very good - 28.2%, good - 25.1%, fair - 19.2%, poor - 13.6%, to very poor - 13.9%. Men with poor to very poor erectile function compared to men with good and very good erectile function had 2.9 the odds of having elevated Factor VII levels (P=0.03), 1.9 times the odds of having elevated vascular cell adhesion molecule (P=0.13) and 2.0 times the odds of having elevated intracellular adhesion molecule (P=0.06) and 2.1 times the odds of having elevated total cholesterol/high-density lipoprotein ratio (P=0.02) comparing the top to bottom quintiles for each atherosclerotic biomarker after multivariate adjustment. Lipoprotein(a), homocysteine, interleukin-6 and tumor necrosis factor receptor, C-reactive protein and fibrinogen were not associated with the degree of erectile function after adjustment. We conclude that selected biomarkers for endothelial function, thrombosis and dyslipidemia but not inflammation are associated with the degree of ED in this cross-sectional analysis. Future studies evaluating the prospective association of ED, endothelial function and cardiovascular disease appear warranted.

*Int J Impot Res. 2006 Aug 17*

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