

LE Magazine July 2008

## JOURNAL ABSTRACTS

### Sun Protection

#### **INTRINSIC AND EXTRINSIC FACTORS IN SKIN AGEING: A REVIEW.**

As the proportion of the ageing population in industrialized countries continues to increase, the dermatological concerns of the aged grow in medical importance. Intrinsic structural changes occur as a natural consequence of ageing and are genetically determined. The rate of ageing is significantly different among different populations, as well as among different anatomical sites even within a single individual. The intrinsic rate of skin ageing in any individual can also be dramatically influenced by personal and environmental factors, particularly the amount of exposure to ultraviolet light. Photodamage, which considerably accelerates the visible ageing of skin, also greatly increases the risk of cutaneous neoplasms. As the population ages, dermatological focus must shift from ameliorating the cosmetic consequences of skin ageing to decreasing the genuine morbidity associated with problems of the ageing skin. A better understanding of both the intrinsic and extrinsic influences on the ageing of the skin, as well as distinguishing the retractable aspects of cutaneous ageing (primarily hormonal and lifestyle influences) from the irretractable (primarily intrinsic ageing), is crucial to this endeavour.

Int J Cosmet Sci. 2008 Apr;30(2):87-95

#### **THE AGE OF SKIN CANCERS.**

Cancer affects two major cell types in the human skin: epithelial cells and melanocytes. Aging and a previous history of ultraviolet light exposure are major risk factors for skin cancers, including basal and squamous cell carcinomas and melanomas. However, melanomas, which are the most deadly of the skin tumors, display two intriguing characteristics: The incidence is increased and the prognosis is worse in males over 60 years as compared with females of the same age. This Perspective discusses possible reasons for age and gender as melanoma risk factors, as well as the need for studies aimed at unraveling the molecular mechanism of such puzzling events.

Sci Aging Knowledge Environ. 2006 May 24;2006(9):pe13

#### **SKIN CANCER TRENDS IN NORTHERN IRELAND AND CONSEQUENCES FOR PROVISION OF DERMATOLOGY SERVICES.**

**BACKGROUND:** The incidence of skin cancer, both melanoma and nonmelanoma skin cancer (NMSC), is rising throughout the world. The evaluation of trends in skin cancer will allow better planning of the future development of skin cancer services. **OBJECTIVES:** Using data collected from the Northern Ireland Cancer Registry (NICR), the incidence of the three major cutaneous cancers, basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (MM), was determined and the workload associated with their management assessed. **METHODS:** The records of patients with a first diagnosis of BCC, SCC or MM occurring between 1993 and 2002 were retrieved from the NICR database. The annual age- and sex-adjusted incidence rates of all three skin cancers were computed per 100,000 person-years by direct standardization according to the European Standard Population. Trends in incidence were estimated by calculating the estimated annual percentage change using Microsoft Excel. For patients registered with the NICR as having BCC, SCC or MM, the number of pathological reports where malignant samples had been examined was counted and then summed to provide the number of specimens examined each year between 1993 and 2004. **RESULTS:** For all three cancers the age-specific rates for both males and females increased with age, except for MM in men aged 75 years and over, where the rates were seen to decrease. Over the 12-year period there was a 62% increase in the overall number of skin cancer samples processed by local pathology laboratories and a 20% increase in the number of patients. These data highlight the fact that many patients will have more than one skin cancer, which reinforces the benefit in collecting data for both patient and sample numbers in order to obtain a true reflection of the workload. The data have also shown that more affluent men and women have higher rates of BCC and MM than their less affluent counterparts. **CONCLUSIONS:** In view of the data presented it is clear that management of NMSC and MM will impose significant demands on services in the years ahead. This will impact on the entire multidisciplinary team. Future planning, in terms of manpower and resources, will prove essential if we are to remain in a position to manage our patients with these malignant tumours appropriately.

### **A POLYPODIUM LEUCOTOMOS EXTRACT INHIBITS SOLAR-SIMULATED RADIATION-INDUCED TNF-ALPHA AND INOS EXPRESSION, TRANSCRIPTIONAL ACTIVATION AND APOPTOSIS.**

In this report, we have examined the molecular basis of the photoprotective effect of a hydrophilic extract of the fern *Polypodium leucotomos* (PL) *in vitro*, using a solar simulator as the source of UV radiation (SSR). We found that pretreatment of human keratinocytes with PL inhibited SSR-mediated increase of tumor necrosis factor (TNF)-alpha and also abrogated nitric oxide (NO) production. Consistent with this, PL blocked the induction of inducible nitric oxide synthase (iNOS) elicited by SSR. In addition, PL inhibited the SSR-mediated transcriptional activation of NF-kappaB and AP1. Finally, we demonstrated that pretreatment with PL exerted a cytoprotective effect against SSR-induced damage, resulting in increased cell survival. Together, these data postulate a multifactor mechanism of protection not exclusively reliant on the antioxidant capability of PL, and strengthen the basic knowledge on the photoprotective effect of this botanical agent.

Exp Dermatol. 2007 Oct;16(10):823-9

### **PHOTOPROTECTIVE PROPERTIES OF A HYDROPHILIC EXTRACT OF THE FERN POLYPODIUM LEUCOTOMOS ON HUMAN SKIN CELLS.**

The effect of a hydrophilic extract of the fern *Polypodium leucotomos* (PLE) has been investigated in terms of photoprotection against UV-induced cell damage. PLE efficiently preserved human fibroblast survival and restored their proliferative capability when the cells were exposed to UVA light. This effect was specific and dose-dependent. Photoprotection was not restricted to fibroblasts, as demonstrated by its effect on survival and proliferation of the human keratinocyte cell line HaCat. Finally, treatment of the cells with PLE prevented UV-induced morphological changes in human fibroblasts, namely disorganisation of F-actin-based cytoskeletal structures, coalescence of the tubulin cytoskeleton and mislocalization of adhesion molecules such as cadherins and integrins. Our *in vitro* results demonstrate the photoprotective effect of PLE on human cells and support its use in the preventive treatment of sunburning and skin pathologies associated with UV-mediated damage.

J Photochem Photobiol B. 2003 Apr;70(1):31-7

### **PHOTOPROTECTIVE ACTIVITY OF ORAL POLYPODIUM LEUCOTOMOS EXTRACT IN 25 PATIENTS WITH IDIOPATHIC PHOTODERMATOSES.**

**BACKGROUND:** The incidences of idiopathic photodermatoses (IP) are increasing and the available therapeutic methods are often inadequate. **AIM:** To evaluate whether, in subjects affected by IP not responding to the usual available therapies, the oral administration of an extract of *Polypodium leucotomos* (PL) could provide an effective photoprotective activity. **METHODS:** 26 patients with polymorphic light eruption and two with solar urticaria were recruited to enter the study. The protocol excluded the use of ultraviolet protection filters or other drugs that could in some way interfere with exposure to light. All patients exposed themselves to sunlight while consuming 480 mg/day of PL orally. The response of the skin to sunlight exposure of 25 evaluable patients was compared with that occurring previously without administration of PL. **RESULTS:** With PL, we observed a relevant and statistically significant reduction of skin reaction and subjective symptoms. The tolerance of the drug has been excellent. **CONCLUSION:** PL extract administration has shown to be an effective and safe method, leading to a significant protection of skin in IP.

Photodermatol Photoimmunol Photomed. 2007 Feb;23(1):46-7

### **POLYPODIUM LEUCOTOMOS EXTRACT: A NUTRACEUTICAL WITH PHOTOPROTECTIVE PROPERTIES.**

Ultraviolet (UV) irradiation causes multifaceted damage to the skin and adjacent tissue layers, and is one of the leading causes of premature skin aging, immunosuppression and carcinogenesis. Photoprotection can be achieved by the use of sunscreens and also by systemically administered compounds that fight the deleterious biological effects of UV exposure, or preferably both. In this review, we summarize the current knowledge on the tissue, cellular and molecular mechanisms underlying the photoprotective effect of *Polypodium leucotomos* fern extract. *P. leucotomos* blocked the deleterious effect of UV irradiation both *in vivo* and *in vitro*. The molecular basis of photoprotection relies on its ability to inhibit free radical generation, prevent photodecomposition of both endogenous photoprotective molecules and DNA, and prevent UV-induced cell death. Its complete loss of toxicity combined with its multifactor protection makes it a valuable tool not only for direct photoprotection, but also as an efficacious adjuvant to phototherapy of various skin diseases.

Drugs Today (Barc). 2007 Jul;43(7):475-85

## **THE ANTIOXIDANT ACTION OF POLYPODIUM LEUCOTOMOS EXTRACT AND KOJIC ACID: REACTIONS WITH REACTIVE OXYGEN SPECIES.**

Two natural products *Polypodium leucotomos* extract (PL) and kojic acid (KA) were tested for their ability to scavenge reactive oxygen species ( $\cdot\text{OH}$ ,  $\text{O}_2^-$ ,  $\text{H}_2\text{O}_2$ ,  $^1\text{O}_2$ ) in phosphate buffer. Hydroxyl radicals were generated by the Fenton reaction, and the rate constants of scavenging were  $1.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  for KA and  $1.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  for PL, similar to that of ethanol ( $1.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). With superoxide anions generated by the xanthine/hypoxanthine system, KA and PL (0.2-1.0 mg/ml) inhibited  $\text{O}_2^-$ -dependent reduction of nitroblue tetrazolium by up to 30 and 31%, respectively. In the detection of  $^1\text{O}_2$  by rose bengal irradiation, PL at 1.0 mg/ml quenched singlet oxygen by 43% relative to azide and KA by 36%. The present study demonstrates that PL showed an antioxidant effect, scavenging three of four reactive oxygen species tested here. Unlike KA, PL did not significantly scavenge hydrogen peroxide.

Braz J Med Biol Res. 2001 Nov;34(11):1487-94

## **POLYPODIUM LEUCOTOMOS EXTRACT INHIBITS TRANS-UROCANIC ACID PHOTOISOMERIZATION AND PHOTODECOMPOSITION.**

In this report, we demonstrate a possible molecular mechanism by which a hydrophilic extract of the leaves of the fern *Polypodium leucotomos* (Fernblock, PL) blocks ultraviolet (UV)-induced skin photodamage. The extract inhibits UVA and UVB light induced photoisomerization of trans-urocanic acid (t-UCA), a common photoreceptor located in the stratum corneum, and also blocks its photodecomposition in the presence of oxidizing reagents such as  $\text{H}_2\text{O}_2$ , and titanium dioxide ( $\text{TiO}_2$ ). PL protects in vitro human fibroblasts from UV-induced death as well. These results suggest the potential of employing the PL extract as a component of sunscreen moistures in order to prevent photodecomposition of t-UCA, to inhibit UV-induced deleterious effects of  $\text{TiO}_2$  and to protect skin cells and endogenous molecules directly involved in skin immunosurveillance.

J Photochem Photobiol B. 2006 Mar 1;82(3):173-9

## **APOPTOSIS AND PATHOGENESIS OF MELANOMA AND NONMELANOMA SKIN CANCER.**

Skin cancers, i.e., basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma, belong to the most frequent tumors. Their formation is based on constitutional and/or inherited factors usually combined with environmental factors, mainly UV-irradiation through long term sun exposure. UV-light can randomly induce DNA damage in keratinocytes, but it can also mutate genes essential for control and surveillance in the skin epidermis. Various repair and safety mechanisms exist to maintain the integrity of the skin epidermis. For example, UV-light damaged DNA is repaired and if this is not possible, the DNA damaged cells are eliminated by apoptosis (sunburn cells). This occurs under the control of the p53 suppressor gene. Fas-ligand (FasL), a member of the tumor necrosis superfamily, which is preferentially expressed in the basal layer of the skin epidermis, is a key surveillance molecule involved in the elimination of sunburn cells, but also in the prevention of cell transformation. However, UV light exposure downregulates FasL expression in keratinocytes and melanocytes leading to the loss of its sensor function. This increases the risk that transformed cells are not eliminated anymore. Moreover, important control and surveillance genes can also be directly affected by UV-light. Mutation in the p53 gene is the starting point for the formation of SCC and some forms of BCC. Other BCCs originate through UV light mediated mutations of genes of the hedgehog signaling pathway which are essential for the maintenance of cell growth and differentiation. The transcription factor Gli2 plays a key role within this pathway, indeed, Gli2 is responsible for the marked apoptosis resistance of the BCCs. The formation of malignant melanoma is very complex. Melanocytes form nevi and from the nevi melanoma can develop through mutations in various genes. Once the keratinocytes or melanocytes have been transformed they re-express FasL which may allow the expanding tumor to evade the attack of immune effector cells. FasL which is involved in immune evasion or genes which govern the apoptosis resistance, e.g., Gli2 could therefore be prime targets to prevent tumor formation and growth. Attempts to silence these genes by RNA interference using gene specific short interfering RNAs (siRNAs) or short hairpin RNAs (shRNAs) have been functionally successful not only in tissue cultures and tumor tissues, but also in a mouse model. Thus, siRNAs and/or shRNAs may become a novel and promising approach to treat skin cancers at an early stage.

Adv Exp Med Biol. 2008;624:283-95

## **PHOTOAGEING: MECHANISM, PREVENTION AND THERAPY.**

Photoageing is the superposition of chronic ultraviolet (UV)-induced damage on intrinsic ageing and accounts for most age-associated changes in skin appearance. It is triggered by receptor-initiated signalling, mitochondrial damage, protein oxidation and telomere-based DNA damage responses. Photodamaged skin displays variable epidermal thickness, dermal elastosis, decreased/fragmented collagen, increased matrix-degrading metalloproteinases, inflammatory infiltrates and vessel ectasia. The development of cosmetically pleasing sunscreens that protect against both UVA and UVB irradiation as well as products such as tretinoin that antagonize the UV signalling pathways leading to photoageing are major steps forward in preventing and reversing photoageing. Improved understanding of the skin's innate UV protective mechanisms has also given rise to several novel

treatment concepts that promise to revolutionize this field within the coming decade. Such advances should not only allow for the improved appearance of skin in middle age and beyond, but also greatly reduce the accompanying burden of skin cancer.

Br J Dermatol. 2007 Nov;157(5):874-87

### **HOW BEST TO HALT AND/OR REVERT UV-INDUCED SKIN AGEING: STRATEGIES, FACTS AND FICTION.**

Once considered mainly a cosmetic issue, photoageing research has long moved to the forefront of investigative dermatology. Besides obvious market pressures, increasing insight into the mechanistic overlap between UV-induced skin cancer and UV-induced skin ageing has contributed to this development. Also, as strategies that work to antagonize intrinsic skin ageing/senescence may also be exploited against photoageing (and vice versa!), it has become an important skin research challenge to dissect both the differences and the overlap mechanisms between these intertwined, yet distinct phenomena. Finally, the current surge in putative 'antiageing' products, devices, and strategies - too many of which boldly promise to fight and/or repair the perils that come along with a lifetime spent in the sun in the absence of convincing evidence of efficacy - makes it particularly pertinent to critically review the available evidence to support often made antiageing claims. The current **CONTROVERSIES** feature, therefore, aimed to provide both guidance through, and critical voices in, the antiageing circus. Here, a panel of experts defines relevant key problems, points the uninaugurated to intriguing aspects of photoageing that one may not have considered before, highlights promising strategies for how best to halt and/or revert it, and spiritedly debates some controversially discussed approaches.

Exp Dermatol. 2008 Mar;17(3):228-40

## Omega-3 Fatty Acids

### **LONG-TERM FISH CONSUMPTION IS ASSOCIATED WITH PROTECTION AGAINST ARRHYTHMIA IN HEALTHY PERSONS IN A MEDITERRANEAN REGION—THE ATTICA STUDY.**

**BACKGROUND:** Dietary habits have long been associated with many manifestations of cardiovascular disease. **OBJECTIVE:** We sought to investigate whether a diet enriched with fish and n-3 fatty acid consumption are associated with changes in the potential duration of the electrical action, as represented by the QT duration on a resting electrocardiogram, in a population-based sample of Greek adults. **DESIGN:** During 2001 and 2002, we randomly enrolled 1514 men (18-87 y old) and 1528 women (18-89 y old) stratified by age and sex distribution (in the 2001 Greek census) from the Attica area, Greece. We studied several demographic, anthropometric, lifestyle, dietary, and bioclinical factors of the participants. Dietary habits (including fish consumption) were evaluated by using a validated food-frequency questionnaire. All subjects underwent electrocardiography with a 12-lead surface, in which, along with several other indexes, QT duration was measured, and the heart rate-corrected QT (QTc) was calculated (corrected by using Bazett's rate). The tested hypothesis was evaluated through multiple linear regression analysis, after control for physical activity status, sex, age, medication intake, and several other potential confounders. **RESULTS:** Compared with fish nonconsumers, those who consumed >300 g fish/wk had a mean 13.6% lower QTc ( $P<0.01$ ). These findings were confirmed after adjustment for age, sex, physical activity status, BMI, smoking habits, intake of nuts, and other confounders. Moreover, compared with fish nonconsumers, those who consumed  $\geq 300$  g fish/wk had a 29.2% lower likelihood of having QTc intervals  $>0.45$  s ( $P=0.03$ ). **CONCLUSIONS:** Long-term consumption of fish is associated with lower QTc interval in free-eating people without any evidence of cardiovascular disease. Thus, fish intake seems to provide antiarrhythmic protection at a population level.

Am J Clin Nutr. 2007 May;85(5):1385-91

### **ANTIARRHYTHMIC EFFECTS OF OMEGA-3 FATTY ACIDS.**

Fish oil, and omega-3 fatty acids in particular, have been found to reduce plasma levels of triglycerides and increase levels of high-density lipoprotein in patients with marked hypertriglyceridemia, and a pharmaceutical-grade preparation has recently received approval from the US Food and Drug Administration to market for this purpose. However, in both bench research studies and clinical trials, evidence for clinically significant antiarrhythmic properties has also been detected in association with omega-3 fatty acid intake. Arguably the most significant finding in this data set was the reduction in the incidence of sudden death in survivors of myocardial infarction in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione trial and the subsequent recommendation for administration of fish oil as part of the postinfarction regimen in Europe. This article reviews in detail the basic and clinical research studies of fish oil as an antiarrhythmic entity, the forms of preparation and/or administration that appear to possess these properties and those that do not, the types of arrhythmias (ventricular ectopy and atrial fibrillation as well as ventricular tachyarrhythmias) that have been beneficially affected by fish oil administration, and the presumed and known mechanisms by which the beneficial actions are exerted.

Am J Cardiol. 2006 Aug 21;98(4A):50i-60i

### **SAFETY CONSIDERATIONS WITH OMEGA-3 FATTY ACID THERAPY.**

It has been suggested that the potential antithrombotic effect of fish oils may theoretically increase the risk for bleeding, which may be a safety concern for individual patients. However, clinical trial evidence has not supported increased bleeding with omega-3 fatty acid intake, even when combined with other agents that might also increase bleeding (such as aspirin and warfarin). Another potential safety concern is the susceptibility of omega-3 fatty acid preparations to undergo oxidation, which contributes to patient intolerance and potential toxicity. Finally, large amounts of fish consumption may result in adverse experiences due to the potential presence of environmental toxins such as mercury, polychlorinated biphenyls, dioxins, and other contaminants. The risks of exposure to environmental toxins and hypervitaminosis with fish consumption are substantially reduced through purification processes used to develop selected concentrated fish oil supplements and prescription preparations. Thus, in choosing which fish oil therapies to recommend, clinicians should be aware of available information to best assess their relative safety, which includes the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) advisory statement regarding fish consumption, the meaning of certain labeling (such as "verification" through the US Pharmacopeia) and the differences in FDA regulatory requirements between nonprescription fish oil supplements and prescription fish oil preparations, and how all of this is important to the optimal treatment of patients.

## **INTERVENTIONS FOR PREVENTING POST-OPERATIVE ATRIAL FIBRILLATION IN PATIENTS UNDERGOING HEART SURGERY.**

**BACKGROUND:** Post-operative atrial fibrillation is a common complication of cardiac surgery and has been associated with increased incidence of other complications including post-operative stroke, increased hospital length of stay and increased cost of hospitalisation. Prevention of atrial fibrillation is a reasonable clinical goal and, consequently, many randomised trials have evaluated the effectiveness of pharmacological and non-pharmacological interventions. We systematically reviewed the literature and prepared meta-analyses to better understand the role and effects of various prophylactic therapies against post-operative atrial fibrillation. **OBJECTIVES:** To assess the effects of pharmacological and non-pharmacological interventions for preventing post-cardiac surgery atrial fibrillation. **SEARCH STRATEGY:** We searched CENTRAL, MEDLINE, EMBASE and CINAHL from earliest achievable date to June 2003. We hand searched references from reports and earlier reviews. We searched abstract books and CD-ROMs from annual scientific meetings of American College of Cardiology, American Heart Association, North American Society of Pacing and Electrophysiology and European Heart Organization between 1997-2003. No language restrictions were applied. **SELECTION CRITERIA:** Randomised controlled trials comparing pharmacological interventions or non-pharmacological interventions with control treatment, placebo or usual care for the prevention of post-operative atrial fibrillation in post-coronary artery bypass grafting or combined CABG and valvular surgery. **DATA COLLECTION AND ANALYSIS:** Two reviewers assessed trial quality and extracted data. Study authors were contacted for additional information. **MAIN RESULTS:** Fifty eight studies were included with a total of 8,565 participants. Interventions included were amiodarone, beta blockers, solatol and pacing. Results favoured treatment for post-operative atrial fibrillation. The data for stroke favoured treatment by a non-significant effect size of 0.81, 95% confidence interval 0.51 to 1.28. Similarly, a positive indication for length of stay was derived but it too was not significant with a weighted mean difference of -0.66, 95% confidence interval -0.95 to -0.37. A positive result for cost of hospitalisation in favour of treatment was achieved, but the statistic is not significant due to low power and large standard deviations: a weighted mean difference of -2717, 95% confidence interval 7,518 to 2,084. Beta-blockers had the greatest magnitude of effect across 28 trials (4074 patients) with an odds ratio (random) of 0.35, 95% confidence interval 0.26 to 0.49. Across all treatment, the odds ratio favoured treatment with a ratio (random) of 0.43, 95% confidence interval 0.37 to 0.51. **REVIEWERS' CONCLUSIONS:** Intervention is favoured across the three pharmacological interventions studied and the one non-pharmacological intervention, pacing. The length of stay data favoured treatment (-0.66, 95% confidence interval -0.95 to -0.37).

Cochrane Database Syst Rev. 2004 Oct 18;(4):CD003611

## **AMIODARONE PROPHYLAXIS FOR ATRIAL FIBRILLATION OF HIGH-RISK PATIENTS AFTER CORONARY BYPASS GRAFTING: A PROSPECTIVE, DOUBLE-BLINDED, PLACEBO-CONTROLLED, RANDOMIZED STUDY.**

**AIMS:** Atrial fibrillation (AF) occurs often in patients after coronary artery bypass grafting (CABG) and can result in increased morbidity and mortality. Previous studies using P-wave signal-averaged electrocardiogram (P-SAECG) have shown that patients with a longer filtered P-wave duration (FPD) have a high risk of AF after CABG. We have shown that patients with an FPD  $>$  or  $=$  124 ms and a root-mean-square voltage of the last 20 ms of the P-wave  $20 <$  or  $=$  3.7 microV have an increased risk of AF after surgery. Accordingly, the aim of this study was to investigate whether or not prophylactic peri-operative administration of amiodarone could reduce the incidence of AF in this high-risk group undergoing CABG identified by P-SAECG. **METHODS AND RESULTS:** In this prospective, double-blinded, placebo-controlled, randomized study, 110 patients received either amiodarone (n = 55) or placebo (n = 55). During CABG, two patients of both groups died. Amiodarone was given as 600 mg oral single dose one day before and from days 2 through 7 after surgery. In addition, amiodarone was also administered intravenously during surgery in a 300-mg bolus for 1 h and as a total maintenance dose of 20 mg/kg weight over 24 h on the first day following surgery. The primary endpoint was the occurrence of AF after CABG. The secondary endpoint was the hospitalization length of stay after CABG. The baseline characteristics were similar in both treatment groups. The incidence of post-operative AF was significantly higher in the placebo group compared with the amiodarone group (85 vs. 34% of patients,  $P < 0.0001$ ). The prophylactic therapy with amiodarone significantly reduced the intensive care ( $1.8 \pm 1.7$  vs.  $2.4 \pm 1.5$  days,  $P = 0.001$ ) and hospitalization length of stay ( $11.3 \pm 3.4$  vs.  $13.0 \pm 4.3$  days,  $P = 0.03$ ). In the amiodarone group, concentrations of amiodarone and desethylamiodarone differed significantly between patients with AF and sinus rhythm (amiodarone:  $0.96 \pm 0.5$  vs.  $0.62 \pm 0.4$  microg/mL,  $P = 0.02$ ; desethylamiodarone:  $0.65 \pm 0.2$  vs.  $0.48 \pm 0.1$  microg/mL,  $P = 0.04$ ). **CONCLUSION:** The incidence of post-operative AF among high-risk patients was significantly reduced by a prophylactic amiodarone treatment resulting in a shorter time of intensive care unit and hospital stay. Our data supports the prophylactic use of amiodarone in peri-operative period in patients at high risk for AF after CABG.

Eur Heart J. 2006 Jul;27(13):1584-91

## **DIETARY ALPHA-LINOLENIC ACID INTAKE AND RISK OF SUDDEN CARDIAC DEATH AND CORONARY HEART DISEASE.**

**BACKGROUND:** Alpha-linolenic acid, an intermediate-chain n-3 fatty acid found primarily in plants, may decrease the risk of fatal

coronary heart disease (CHD) through a reduction in fatal ventricular arrhythmias and sudden cardiac death (SCD). **METHODS AND RESULTS:** We prospectively examined the association between dietary intake of alpha-linolenic acid assessed via updated food-frequency questionnaires and the risk of SCD, other fatal CHD, and nonfatal myocardial infarction (MI) among 76,763 women participating in the Nurses' Health Study who were free from cancer and completed a dietary questionnaire at baseline in 1984. During 18 years of follow-up, we identified 206 SCDs, 641 other CHD deaths, and 1604 nonfatal MIs. After controlling for coronary risk factors and other fatty acids, including long-chain n-3 fatty acids, the intake of alpha-linolenic acid was inversely associated with the risk of SCD (P for trend, 0.02) but not with the risk of other fatal CHD or nonfatal MI. Compared with women in the lowest quintile of alpha-linolenic acid intake, those in the highest 2 quintiles had a 38% to 40% lower SCD risk. This inverse relation with SCD risk was linear and remained significant even among women with high intakes of long-chain n-3 fatty acids. **CONCLUSIONS:** These prospective data suggest that increasing dietary intake of alpha-linolenic acid may reduce the risk of SCD but not other types of fatal CHD or nonfatal MI in women. The specificity of the association between alpha-linolenic acid and SCD supports the hypothesis that these n-3 fatty acids may have antiarrhythmic properties.

Circulation. 2005 Nov 22;112(21):3232-8

### **A BRIEF HISTORY OF SUDDEN CARDIAC DEATH AND ITS THERAPY.**

At the end of the 19th century, there was both experimental and clinical evidence that coronary artery obstruction causes ventricular fibrillation and sudden death and that fibrillation could be terminated by electric shocks. The dominant figure at that time was McWilliam, who in 1923 complained that "little attention was given to the new view for many years." This remained so for many decades. It was not until the 1960s that the medical profession became aware of the magnitude of the problem of sudden death and began to install coronary care units where arrhythmias could be monitored and prompt defibrillation could be delivered. This approach was pioneered by Julian in 1961. Milestones that allowed this development were open-chest defibrillation by Beck, closed-chest defibrillation by Zoll, cardiac massage by Kouwenhoven et al., and development of the DC defibrillator by Lown. In 1980, Mirowski et al. implanted the first implantable cardioverter defibrillator (ICD) in a patient. Thereafter, the use of the ICD increased exponentially. Several randomized trials, largely in patients with coronary artery disease and left ventricular dysfunction or in patients with documented lethal arrhythmias, showed beyond doubt that the ICD is superior to antiarrhythmic drug therapy in preventing sudden death, although a number of trials showed no effect. Trials on antiarrhythmic drugs were disappointing. Sodium channel blockers and "pure" potassium channel blockers actually increase mortality, calcium channel blockers have no effect, and, although amiodarone reduces arrhythmic death, it had no effect on total mortality in the 2 largest trials. Only the beta-blockers have been proven to reduce the incidence of sudden death, but their effect appears not to be related to the suppression of arrhythmias but rather to the reduction in sinus rate. Drugs that prevent ischemic events, or lessen their impact, such as anticoagulants, statins, angiotensin-converting enzyme inhibitors, and aldosterone antagonists, all reduce the incidence of sudden death.

Pharmacol Ther. 2003 Oct;100(1):89-99

### **DIETARY FISH OIL REDUCES THE OCCURRENCE OF EARLY AFTERDEPOLARIZATIONS IN PIG VENTRICULAR MYOCYTES.**

Fish oil reduces sudden cardiac death in post myocardial infarction patients. Life-threatening arrhythmias in heart failure are associated with repolarization abnormalities leading to EAD(1) formation. We examined the effects of incorporated fish oil omega3-PUFAs(2) on EAD formation in pig myocytes. Pigs were fed a diet rich in fish oil or sunflower oil (control) for 8 weeks. Myocytes were isolated by enzymatic dissociation and patch-clamped. Susceptibility to EAD formation was tested using E4031 (5 microM), a blocker of I(Kr). The fish oil diet in pigs resulted in increased incorporation of omega3-PUFAs in the sarcolemma of the myocytes compared to the control diet and caused a reduced occurrence of E4031-induced EADs in pig myocytes. A shorter action potential, a reduced action potential prolongation in response to E-4031 and a reduced reactivation of I(Ca,L) by omega3-PUFAs may explain the observed reduction in EADs. A diet rich in fish oil protects against EAD formation.

J Mol Cell Cardiol. 2006 Nov;41(5):914-7

### **THE RELATIONSHIP BETWEEN FISH CONSUMPTION AND STROKE INCIDENCE. THE NHANES I EPIDEMIOLOGIC FOLLOW-UP STUDY (NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY).**

**OBJECTIVE:** To assess the level of fish consumption as a risk factor for stroke. **METHODS:** Participants were members of the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study, a longitudinal cohort study of a national sample. Included in this analysis were white and black women and men aged 45 to 74 years when examined in 1971 through 1975 who did not report a history of stroke at that time. Average follow-up for survivors was 12 years (maximum, 16 years). The main outcome measure was incident stroke (fatal and nonfatal). Fish consumption at baseline was obtained from a 3-month food frequency questionnaire. **RESULTS:** White women aged 45 to 74 years who consumed fish more than once a week had an age-adjusted risk of stroke incidence only about half that of women who never consumed fish. This effect persisted after controlling for multiple stroke risk variables (relative risk, 0.55;95% confidence interval [CI], 0.32 to 0.93). Fish consumption more than once a

week compared with never was not associated with age-adjusted stroke risk in white men aged 45 to 74 years (relative risk, 0.85;95%CI,0.49 to 1.46). In black women and men combined aged 45 to 74 years, any fish consumption compared with never was significantly associated with reduced adjusted stroke risk (relative risk, 0.51;95%CI,0.30 to 0.88).

Arch Intern Med. 1996 Mar 11;156(5):537-42

### **EFFECTS OF DIETARY FISH OIL SUPPLEMENTATION ON PLATELET AGGREGABILITY AND PLATELET MEMBRANE FLUIDITY IN NORMOLIPEMIC SUBJECTS WITH AND WITHOUT HIGH PLASMA LP(A) CONCENTRATIONS.**

The purpose of this study was to compare the relative effect of n-3 fatty acids on plasma lipids and platelet function in normolipemic subjects (n = 8) with plasma Lp(a) levels greater than 30 mg/dl and normolipemic subjects (n = 7) without detectable plasma Lp(a) concentrations. Six weeks of dietary supplementation (3.8 g EPA and 2.9 g DHA/d) significantly reduced (P less than 0.005) plasma TGs in both groups whereas no changes of plasma TC, LDL-C, HDL-C, and Lp(a), respectively, were found. Collagen- or thrombin-stimulated platelet aggregation and collagen- or thrombin-induced TXB<sub>2</sub> generation from platelets decreased by approx. 45% in Lp(a)-negative and Lp(a)-positive platelet donors after a 6 week dietary intake. Four more weeks without n-3 supplementation restored the pretreatment values of TGs, platelet aggregability and TXB<sub>2</sub> release. The biophysical properties of platelets from normolipemics with and without high plasma Lp(a) concentrations revealed a similar structural order of platelets at 37 degrees C using DPH, TMA-DPH, or 6-AS as fluorescent probes. Also similar temperature-dependent changes in platelet fluidity from 37 degrees C to 17 degrees C were observed in platelet preparations from Lp(a)-positive and Lp(a)-negative subjects. However, no subtle changes in the structural order of platelets due to nutrient intakes were found in all subjects (n = 15, 19-28 yrs) using fluorescence polarization technique. The present data suggest a similar in vitro platelet behaviour from normolipemic subjects with and without high plasma levels of Lp(a) (which is considered a risk for premature atherosclerosis) in contrast to platelet aggregability and platelet fluidity in certain hyperlipidemic stages.

Atherosclerosis. 1991 Jun;88(2-3):193-201

**Cognitive impairment****CONVERSION OF MILD COGNITIVE IMPAIRMENT TO DEMENTIA IN ELDERLY SUBJECTS: A PRELIMINARY STUDY IN A MEMORY AND COGNITIVE DISORDER UNIT.**

Prevalence and incidence of predementia syndromes vary as a result of different diagnostic criteria, as well as different sampling and assessment procedures. Mild cognitive impairment (MCI) is thought to be a prodromal phase of dementia and therefore highly predictive of subsequent conversion. The aim of our study was to investigate the risk of conversion to dementia for different MCI subtypes diagnosed according to standardized and recently revised criteria (amnestic; impairment of memory plus other cognitive domains; nonamnestic). Participants were recruited among the 2,866 patients referring to the Memory and Cognitive Disorders Unit of the Local Health Unit of Bologna, Maggiore Hospital, between October 2000 and February 2006. In this preliminary study we analyzed data from 52 elderly outpatients with a diagnosis of MCI and a mean follow-up of 1.21 $\pm$ 0.61 years (range 0.23-3.10 years). Mean age was 72.8 $\pm$ 6.6 years, males were 61.5%. Mean baseline mini mental state examination (MMSE) score was 27.1 $\pm$ 1.5. There were 15 incident cases of dementia (28.8%), with Alzheimer's disease (AD) accounting for 53.3% of all cases, AD with cerebrovascular disease for 33.4% and fronto-temporal dementia for 13.3%. Overall rate of conversion was 23.8 per 100 person-years. During the same follow-up period, 53.8% of participants remained stable and 17.3% reverted to normal. Rates of conversion for the specific MCI subtypes were 38 per 100 person-years for amnestic MCI, 20 per 100 person-years for non-amnestic MCI, and 16 per 100 person-years for memory plus other cognitive domains MCI. With respect to non-converters, converters were generally older (76.1 $\pm$ 4.2 vs. 71.5 $\pm$ 7.0 years,  $p=0.021$ ), had a lower MMSE score (26.4 $\pm$ 1.66 vs. 27.4 $\pm$ 1.4,  $p=0.035$ ) and a higher prevalence of atrophy at neuroimaging (73.7% vs. 42.4%,  $p=0.047$ ). Moreover, with respect to non-converters, converters tended to have higher serum high density lipoprotein (HDL) levels, and lower serum folate levels. No difference was observed for the other study variables, included MCI subtype. Our findings suggest that the current definitions for MCI subtypes, particularly those referring to individuals with multiple or non-amnestic cognitive impairment, include a substantial number of individuals who may not progress to dementia. The possible role of cortical atrophy and low folate in the conversion from MCI to dementia could have important implications, because both conditions are easily identifiable. Moreover, low folate status is potentially amenable to therapeutic options. Although discouraging with respect to the clinical usefulness of currently available MCI criteria, our results raise the possibility that defining a protocol of multiple clinical risk factors may be useful in identifying MCI individuals at increased risk of conversion.

Arch Gerontol Geriatr. 2007;44 Suppl 1:233-41

**EVIDENCE OF INCREASED OXIDATIVE DAMAGE IN SUBJECTS WITH MILD COGNITIVE IMPAIRMENT.**

**OBJECTIVE:** To determine if increased levels of oxidative damage are present in the brains of persons with mild cognitive impairment (MCI), a condition that often precedes Alzheimer disease (AD). **METHODS:** The authors assessed the amount of protein carbonyls, thiobarbituric acid-reactive substances (TBARS), and malondialdehyde in the superior and middle temporal gyri (SMTG) and cerebellum of short postmortem interval and longitudinally evaluated normal subjects and those with MCI and early AD. **RESULTS:** Elevated levels of protein carbonyls (approximately 25%), malondialdehyde (approximately 60%), and TBARS (approximately 210%) were observed in the SMTG of individuals with MCI and early AD vs normal control subjects. The elevation in TBARS was associated with the numbers of neuritic but not diffuse plaques. Levels of protein carbonyls increased as delayed verbal memory performance declined. **CONCLUSION:** Oxidative damage occurs in the brain of subjects with mild cognitive impairment, suggesting that oxidative damage may be one of the earliest events in the onset and progression of Alzheimer disease.

Neurology. 2005 Apr 12;64(7):1152-6

**OXIDATIVE DAMAGE IN MILD COGNITIVE IMPAIRMENT AND EARLY ALZHEIMER'S DISEASE.**

Increasing evidence supports a role for oxidative damage in the pathogenesis of Alzheimer's disease (AD). Multiple studies show significantly increased levels of lipid peroxidation and protein, DNA, and RNA oxidation in vulnerable regions of the brain of patients with late-stage AD (LAD). More recent studies of patients with amnestic mild cognitive impairment (MCI), the earliest clinical manifestation of AD, show similar patterns of oxidative damage. These observations suggest that oxidative damage to critical biomolecules occurs early in the pathogenesis of AD and precedes pronounced neuropathologic alterations. Because oxidative damage begins early in the progress of the disease, it represents a potential therapeutic target for slowing the onset and progression of AD.

### **(R)-ALPHA-LIPOIC ACID REVERSES THE AGE-RELATED LOSS IN GSH REDOX STATUS IN POST-MITOTIC TISSUES: EVIDENCE FOR INCREASED CYSTEINE REQUIREMENT FOR GSH SYNTHESIS.**

Age-related depletion of GSH levels and perturbations in its redox state may be especially deleterious to metabolically active tissues, such as the heart and brain. We examined the extent and the mechanisms underlying the potential age-related changes in cerebral and myocardial GSH status in young and old F344 rats and whether administration of (R)-alpha-lipoic acid (LA) can reverse these changes. Our results show that GSH/GSSG ratios in the aging heart and the brain declined by 58 and 66% relative to young controls, respectively ( $p < 0.001$ ). Despite a consistent loss in GSH redox status in both tissues, only cerebral GSH levels declined with age ( $p < 0.001$ ). To discern the potential mechanisms underlying this differential loss, the levels and the activities of gamma-glutamylcysteine ligase (GCL) and cysteine availability were determined. There were no significant age-related changes in substrate or enzyme levels, or GCL activity when saturating amounts of substrates were provided. However, kinetic analysis of GCL in brains of old rats displayed a significant increase ( $p < 0.05$ ) in the apparent  $[K_m]$  for cysteine ( $K_m \text{ cys}$ ) vs. young rats ( $84.3 \pm 25.4$  vs.  $179.0 \pm 49.0$ ; young and old, respectively), resulting in a 40% loss in apparent catalytic turnover of the enzyme. Thus, the age-related decline in total GSH appears to be mediated, in part, by a general decrement in GCL catalytic efficiency. Treating old rats with LA (40 mg/kg body wt; by i.p.) markedly increased tissue cysteine levels by 54% 12 h following treatment and subsequently restored the cerebral GSH levels. Moreover, LA improved the age-related changes in the tissue GSH/GSSG ratios in both heart and the brain. These results demonstrate that LA is an effective agent to restore both the age-associated decline in thiol redox ratio as well as increase cerebral GSH levels that otherwise decline with age.

Arch Biochem Biophys. 2004 Mar 1;423(1):126-35

### **L-CARNITINE AND DL-ALPHA-LIPOIC ACID REVERSE THE AGE-RELATED DEFICIT IN GLUTATHIONE REDOX STATE IN SKELETAL MUSCLE AND HEART TISSUES.**

In the present study, the glutathione redox system was evaluated as a function of age in rat heart and muscle. A decline in reduced glutathione (GSH) levels is associated with aging and many age-related diseases. The objective of this study was to determine whether L-carnitine and DL-alpha-lipoic acid could compensate for GSH depletion in protection against oxidative insults. In this study we determined reduced glutathione, oxidized glutathione (GSSG), glutathione peroxidase (GPx), glutathione reductase (GR), and glucose-6-phosphate dehydrogenase (G6PDH) in skeletal muscle and heart of young and aged rats. We also calculated GSH/GSSG molar ratio and glutathione redox system. GSH levels were significantly lowered in aged rats than young rats. Conversely, GSSG levels were significantly high in aged rats. GSH/GSSG molar ratio and redox index were found to be decreased in aged rats. The activities of GPx, GR, and G6PDH were found to be decreased in aged rats when compared with young rats. Supplementation of carnitine and lipoic acid to aged rats significantly increased the GSH levels thereby increasing the activity of GPx, GR, and G6PDH in skeletal muscle and heart of aged rats. In conclusion, our study suggests that supplementation of carnitine and lipoic acid to aged rats improves the glutathione redox system.

Mech Ageing Dev. 2004 Jul;125(7):507-12

### **DELAYING BRAIN MITOCHONDRIAL DECAY AND AGING WITH MITOCHONDRIAL ANTIOXIDANTS AND METABOLITES.**

Mitochondria decay with age due to the oxidation of lipids, proteins, RNA, and DNA. Some of this decay can be reversed in aged animals by feeding them the mitochondrial metabolites acetylcarnitine and lipoic acid. In this review, we summarize our recent studies on the effects of these mitochondrial metabolites and mitochondrial antioxidants (alpha-phenyl-N-t-butyl nitron and N-t-butyl hydroxylamine) on the age-associated mitochondrial decay of the brain of old rats, neuronal cells, and human diploid fibroblast cells. In feeding studies in old rats, these mitochondrial metabolites and antioxidants improve the age-associated decline of ambulatory activity and memory, partially restore mitochondrial structure and function, inhibit the age-associated increase of oxidative damage to lipids, proteins, and nucleic acids, elevate the levels of antioxidants, and restore the activity and substrate binding affinity of a key mitochondrial enzyme, carnitine acetyltransferase. These mitochondrial metabolites and antioxidants protect neuronal cells from neurotoxin- and oxidant-induced toxicity and oxidative damage; delay the normal senescence of human diploid fibroblast cells, and inhibit oxidant-induced acceleration of senescence. These results suggest a plausible mechanism: with age, increased oxidative damage to proteins and lipid membranes, particularly in mitochondria, causes a deformation of structure of enzymes, with a consequent decrease of enzyme activity as well as substrate binding affinity for their substrates; an increased level of substrate restores the velocity of the reaction and restores mitochondrial function, thus delaying mitochondrial decay and aging. This loss of activity due to coenzyme or substrate binding appears to be true for a number of other enzymes as well, including mitochondrial complex III and IV.

Ann N Y Acad Sci. 2002 Apr;959:133-66

## **META-ANALYSIS OF DOUBLE BLIND RANDOMIZED CONTROLLED CLINICAL TRIALS OF ACETYL-L-CARNITINE VERSUS PLACEBO IN THE TREATMENT OF MILD COGNITIVE IMPAIRMENT AND MILD ALZHEIMER'S DISEASE.**

The efficacy of acetyl-L-carnitine (gamma-trimethyl- beta-acetylbutyrobetaine (Alcar) in mild cognitive impairment (MCI) and mild (early) Alzheimer's disease (AD) was investigated with a meta-analysis of double-blind, placebo-controlled prospective, parallel group comparison studies of at least 3 months duration. The duration of the studies was 3, 6 or 12 months and the daily dose varied between studies from 1.5-3.0 g/day. An effect size was calculated to reflect the results of the variety of measures used in the studies grouped into the categories of clinical tests and psychometric tests. The effect sizes from the categories were integrated into an overall summary effect size. The effect size for the Clinical Global Impression of Change (CGI-CH) was calculated separately. Meta-analysis showed a significant advantage for Alcar compared to placebo for the integrated summary effect [ES =0.201, 95% confidence interval (CI)=0.107-0.295] and CGI-CH (ES =0.32, 95% CI=0.18-0.47). The beneficial effects were seen on both the clinical scales and the psychometric tests. The advantage for Alcar was seen by the time of the first assessment at 3 months and increased over time. Alcar was well tolerated in all studies.

Int Clin Psychopharmacol. 2003 Mar;18(2):61-71

## **DENDRITIC SPINE LOSS IN HIPPOCAMPUS OF AGED RATS. EFFECT OF BRAIN PHOSPHATIDYLSERINE ADMINISTRATION.**

Dendritic spine density of pyramidal cells in region CA1 of the hippocampus has been evaluated in young (3 months), old (27 months) and old phosphatidylserine (BC-PS)-treated rats. BC-PS (50 mg/kg, suspended in tap water) was administered daily, starting at the age of 3 months until 27 months. Spine density was analyzed on Golgi-stained pyramidal neurons by a computerized analysis system. In 27-month-old rats, spine density showed with respect to 3-month-old animals, a significant decrease in both basal and apical dendrites ( $p$  less than 0.01; one-way ANOVA), with a mean loss of 12.11% in the basal dendrites and of 10.64% in the apical ones. In 27-month-old rats treated with BC-PS, values of spine density were not statistically different when compared to those of 3-month-old animals. The mechanisms underlying the beneficial effect of BC-PS treatment on neuronal connectivity might be explained on the basis of its pharmacological actions on neuronal membranes [9], neurotransmission [43] and/or interaction with NGF [7].

Neurobiol Aging. 1987 Nov-Dec;8(6):501-10

## **COGNITIVE DECLINE IN THE ELDERLY: A DOUBLE-BLIND, PLACEBO-CONTROLLED MULTICENTER STUDY ON EFFICACY OF PHOSPHATIDYLSERINE ADMINISTRATION.**

This double-blind study assesses the therapeutic efficacy and the safety of oral treatment with phosphatidylserine (BC-PS) vs placebo (300 mg/day for 6 months) in a group of geriatric patients with cognitive impairment. A total of 494 elderly patients (age between 65 and 93 years), with moderate to severe cognitive decline, according to the Mini Mental State Examination and Global Deterioration Scale, were recruited in 23 Geriatric or General Medicine Units in Northeastern Italy. Sixty-nine patients dropped out within the 6-month trial period. Patients were examined just before starting therapy, and 3 and 6 months thereafter. The efficacy of treatment compared to placebo was measured on the basis of changes occurring in behavior and cognitive performance using the Plutchik Geriatric Rating Scale and the Buschke Selective Reminding Test. Statistically significant improvements in the phosphatidylserine-treated group compared to placebo were observed both in terms of behavioral and cognitive parameters. In addition, clinical evaluation and laboratory tests demonstrated that BC-PS was well tolerated. These results are clinically important since the patients were representative of the geriatric population commonly met in clinical practice.

Aging (Milano). 1993 Apr;5(2):123-33

## **GLUTATHIONE METABOLISM DURING AGING AND IN ALZHEIMER DISEASE.**

The concentration of glutathione (GSH), the most abundant intracellular nonprotein thiol and important antioxidant, declines with age and in some age-related diseases. The underlying mechanism, however, is not clear. The previous studies from our laboratory showed that the age-dependent decline in GSH content in Fisher 344 rats was associated with a downregulation of glutamate cysteine ligase (GCL), the rate-limiting enzyme in de novo GSH synthesis. Our recent studies further indicated that the activity and mRNA content of glutathione synthase (GS), which catalyzes the second reaction in de novo GSH synthesis, were also decreased with age in some tissues. No age-associated change was observed in glutathione reductase or gamma-glutamyl transpeptidase activities. Also, although GSH content declined with age in both male and female mice, male mice experienced more dramatic age-associated decline in many tissues/organs than female mice. Furthermore, we found that GSH content was significantly decreased in the red blood cells from male Alzheimer disease patients, which was associated with decreases in GCL and GS activities. Finally, we showed that estrogen increased GSH content, GS and GR activities, and GCL gene expression in the liver of both male and female mice. Taken together, our results suggest that (1) GCL plays a critical role in maintaining GSH homeostasis under both physiological and pathological conditions; (2) decreased GSH content may be

involved in AD pathology in humans; and (3) estrogen increases GSH content in mice by multiple mechanisms.

Ann N Y Acad Sci. 2004 Jun;1019:346-9

### **PROTECTIVE EFFECT OF RESVERATROL ON BETA-AMYLOID-INDUCED OXIDATIVE PC12 CELL DEATH.**

Beta-amyloid peptide is considered to be responsible for the formation of senile plaques that accumulate in the brains of patients with Alzheimer's disease. There has been compelling evidence supporting the idea that beta-amyloid-induced cytotoxicity is mediated through the generation of reactive oxygen intermediates (ROIs). Considerable attention has been focused on identifying phytochemicals that are able to scavenge excess ROIs, thereby protecting against oxidative stress and cell death. Resveratrol (3,5,4'-trihydroxy-trans-stilbene), a phytoalexin found in the skin of grapes, has strong antioxidative properties that have been associated with the protective effects of red wine consumption against coronary heart disease ("the French paradox"). In this study, we have investigated the effects of resveratrol on beta-amyloid-induced oxidative cell death in cultured rat pheochromocytoma (PC12) cells. PC12 cells treated with beta-amyloid exhibited increased accumulation of intracellular ROI and underwent apoptotic death as determined by characteristic morphological alterations and positive in situ terminal end-labeling (TUNEL staining). Beta-amyloid treatment also led to the decreased mitochondrial membrane potential, the cleavage of poly (ADP-ribose)polymerase, an increase in the Bax/Bcl-X(L) ratio, and activation of c-Jun N-terminal kinase. Resveratrol attenuated beta-amyloid-induced cytotoxicity, apoptotic features, and intracellular ROI accumulation. Beta-amyloid transiently induced activation of NF-kappaB in PC12 cells, which was suppressed by resveratrol pretreatment.

Free Radic Biol Med. 2003 Apr 15;34(8):1100-10

## Testosterone

### **ENDOGENOUS TESTOSTERONE AND MORTALITY DUE TO ALL CAUSES, CARDIOVASCULAR DISEASE, AND CANCER IN MEN: EUROPEAN PROSPECTIVE INVESTIGATION INTO CANCER IN NORFOLK (EPIC-NORFOLK) PROSPECTIVE POPULATION STUDY.**

**BACKGROUND:** The relation between endogenous testosterone concentrations and health in men is controversial. **METHODS AND RESULTS:** We examined the prospective relationship between endogenous testosterone concentrations and mortality due to all causes, cardiovascular disease, and cancer in a nested case-control study based on 11,606 men aged 40 to 79 years surveyed in 1993 to 1997 and followed up to 2003. Among those without prevalent cancer or cardiovascular disease, 825 men who subsequently died were compared with a control group of 1,489 men still alive, matched for age and date of baseline visit. Endogenous testosterone concentrations at baseline were inversely related to mortality due to all causes (825 deaths), cardiovascular disease (369 deaths), and cancer (304 deaths). Odds ratios (95% confidence intervals) for mortality for increasing quartiles of endogenous testosterone compared with the lowest quartile were 0.75 (0.55 to 1.00), 0.62 (0.45 to 0.84), and 0.59 (0.42 to 0.85), respectively ( $P < 0.001$  for trend after adjustment for age, date of visit, body mass index, systolic blood pressure, blood cholesterol, cigarette smoking, diabetes mellitus, alcohol intake, physical activity, social class, education, dehydroepiandrosterone sulfate, androstenediol glucuronide, and sex hormone binding globulin). An increase of 6 nmol/L serum testosterone (approximately 1 SD) was associated with a 0.81 (95% confidence interval 0.71 to 0.92,  $P < 0.01$ ) multivariable-adjusted odds ratio for mortality. Inverse relationships were also observed for deaths due to cardiovascular causes and cancer and after the exclusion of deaths that occurred in the first 2 years. **CONCLUSIONS:** In men, endogenous testosterone concentrations are inversely related to mortality due to cardiovascular disease and all causes. Low testosterone may be a predictive marker for those at high risk of cardiovascular disease.

Circulation. 2007 Dec 4;116(23):2694-701

### **RISKS OF TESTOSTERONE REPLACEMENT THERAPY IN AGEING MEN.**

Testosterone has been available to practitioners for several decades. However, testosterone prescriptions have increased in recent years partly because of the introduction of newer delivery systems that are topical and have good bioavailability. In the US alone, approximately 2 million prescriptions for testosterone were written in 2002. This represents a 30% increase from 2001 and a 170% increase from 1999. There has also been a 500% increase in prescription sales in the past 10 years. The rise in prescriptions may be in part due to the increasing recognition of hypogonadism in ageing males or andropause. Treatment relating to hypogonadism has relieved symptoms and improved the quality of life of many individuals. Epidemiological studies point toward an association with increased morbidity and mortality, with low testosterone states in ageing males. For example, there is a higher prevalence of depression, coronary heart disease, osteoporosis, fracture rates, frailty and even dementia with low testosterone states. Recently, there have been some concerns raised regarding the long-term safety of testosterone replacement therapy (TRT) from the Institute of Medicine. Current evidence suggests no causal relationship between prostate cancer and physiological dosing of testosterone, especially with careful selection and monitoring of patients. Cardiovascular risks have, overall, been neutral, although suggestions have been made that there are positive vasodilatory properties with testosterone. Mild erythrocytosis can be a common side effect of TRT, but thromboembolic events have rarely been reported in the literature. This paper addresses the evidence to date regarding the safety aspects of TRT. The medical-legal implications of TRT for men at this point in time is also discussed.

Expert Opin Drug Saf. 2004 Nov;3(6):599-606

### **TESTOSTERONE AND AGEING: WHAT HAVE WE LEARNED SINCE THE INSTITUTE OF MEDICINE REPORT AND WHAT LIES AHEAD?**

A 2003 report by the Institute of Medicine (IOM) surveyed the literature on the benefits and risks of testosterone replacement therapy in older men and identified knowledge gaps and research needs. This review summarises some key studies published since the IOM report. The possible relationship of testosterone to risk of prostate cancer remains a concern; however, no new evidence has emerged to suggest that testosterone replacement therapy increases the risk. Recent studies have demonstrated that hypogonadism in men may be more prevalent than previously thought, is strongly associated with metabolic syndrome, and may be a risk factor for type 2 diabetes and cardiovascular disease. Clinical studies have shown that testosterone replacement therapy in hypogonadal men improves metabolic syndrome indicators and cardiovascular risk factors. Maintaining testosterone

concentrations in the normal range has been shown to contribute to bone health, lean muscle mass, and physical and sexual function, suggesting that testosterone replacement therapy may help to prevent frailty in older men. Based on current knowledge, testosterone replacement therapy is unlikely to pose major health risks in patients without prostate cancer and may offer substantial health benefits. Larger, longer-term randomised studies are needed to fully establish the effects of testosterone replacement therapy.

Int J Clin Pract. 2007 Apr;61(4):622-32

### **TESTOSTERONE AND ATHEROSCLEROSIS IN AGING MEN: PURPORTED ASSOCIATION AND CLINICAL IMPLICATIONS.**

Two of the strongest independent risk factors for coronary heart disease (CHD) are increasing age and male sex. Despite a wide variance in CHD mortality between countries, men are consistently twice as likely to die from CHD than their female counterparts. This sex difference has been attributed to a protective effect of female sex hormones, and a deleterious effect of male sex hormones, upon the cardiovascular system. However, little evidence suggests that testosterone exerts cardiovascular harm. In fact, serum levels of testosterone decline with age, and low testosterone is positively associated with other cardiovascular risk factors. Furthermore, testosterone exhibits a number of potential cardioprotective actions. For example, testosterone treatment is reported to reduce serum levels of the pro-inflammatory cytokines interleukin (IL)-1beta and tumor necrosis factor (TNF)-alpha, and to increase levels of the anti-inflammatory cytokine IL-10; to reduce vascular cell adhesion molecule (VCAM)-1 expression in aortic endothelial cells; to promote vascular smooth muscle and endothelial cell proliferation; to induce vasodilatation and to improve vascular reactivity, to reduce serum levels of the pro-thrombotic factors plasminogen activator inhibitor (PAI)-1 and fibrinogen; to reduce low-density lipoprotein-cholesterol (LDL-C); to improve insulin sensitivity; and to reduce body mass index and visceral fat mass. These actions of testosterone may confer cardiovascular benefit since testosterone therapy reduces atheroma formation in cholesterol-fed animal models, and reduces myocardial ischemia in men with CHD. Consequently, an alternative hypothesis is that an age-related decline in testosterone contributes to the atherosclerotic process. This is supported by recent findings, which suggest that as many as one in four men with CHD have serum levels of testosterone within the clinically hypogonadal range. Consequently, restoration of serum levels of testosterone via testosterone replacement therapy could offer cardiovascular, as well as other, clinical advantages to these individuals.

Am J Cardiovasc Drugs. 2005;5(3):141-54

### **TESTOSTERONE, DIABETES MELLITUS, AND THE METABOLIC SYNDROME.**

Metabolic syndrome is characterized by insulin insensitivity, central obesity dyslipidemia, and hypertension. It is recognized as a risk factor for cardiovascular disease in men; by the time metabolic syndrome is diagnosed, however, most men already have entrenched cardiovascular disease. A reliable early warning sign is needed to alert physicians to those at risk for metabolic syndrome and cardiovascular disease. Low serum testosterone level has emerged as a reliable prognosticator of metabolic syndrome in men whose testosterone deficiency is genetic (Klinefelter syndrome), iatrogenic following surgery for testicular cancer, pharmacologically induced by gonadotropin-releasing hormone during prostate cancer treatment, or a natural consequence of aging. One third of men with type 2 diabetes mellitus are now recognized as testosterone deficient. Emerging evidence suggests that testosterone therapy may be able to reverse some aspects of metabolic syndrome.

Curr Urol Rep. 2007 Nov;8(6):467-71

### **ANDROGEN DEFICIENCY, DIABETES, AND THE METABOLIC SYNDROME IN MEN.**

**PURPOSE OF REVIEW:** The burden of androgen deficiency in men with diabetes and the metabolic syndrome has become increasingly apparent in population-based studies. This article focuses on the mechanisms underlying the interdependent relationship between these conditions. **RECENT FINDINGS:** Various definitions of hypogonadism, the metabolic syndrome and diabetes have been proposed and are used in the literature. Cross-sectional studies have found that between 20 and 64% of men with diabetes have hypogonadism, with higher prevalence rates found in the elderly. Hypogonadism can be a risk factor for the development of diabetes and the metabolic syndrome through various mechanisms including changes in body composition; androgen receptor polymorphisms; glucose transport; and reduced antioxidant effect. Conversely, diabetes and the metabolic syndrome can be risk factors for hypogonadism through some similar but mostly distinct mechanisms, such as increased body weight; decreased sex hormone binding globulin levels; suppression of gonadotrophin release or Leydig cell testosterone production; cytokine-mediated inhibition of testicular steroid production; and increased aromatase activity contributing to relative estrogen excess. **SUMMARY:** The relationship between diabetes, the metabolic syndrome and androgen deficiency is complex. Testosterone supplementation, by either oral or intramuscular routes and through exogenous or endogenous delivery, has a promising role in this population although further clinical trials are needed.

Curr Opin Endocrinol Diabetes Obes. 2007 Jun;14(3):226-34

## EFFECT OF TESTOSTERONE ON INSULIN SENSITIVITY IN MEN WITH IDIOPATHIC HYPOGONADOTROPIC HYPOGONADISM.

**OBJECTIVE:** To assess the presence of insulin resistance (IR) among a homogeneous cohort of male patients with idiopathic hypogonadotropic hypogonadism (IHH) and to investigate the effects of testosterone therapy on IR in this specific group.

**METHODS:** Twenty-four male patients with untreated IHH and 20 age-, sex-, and weight-matched eugonadal healthy control subjects were recruited for the study. Plasma glucose, plasma insulin, total and free testosterone, follicle-stimulating hormone, luteinizing hormone, estradiol, and sex hormone-binding globulin levels were measured in fasting blood samples, and biochemical and hormonal analyses were performed for all study participants. IR was calculated by the homeostasis model assessment of insulin resistance (HOMA-IR) formula and the quantitative insulin sensitivity check index (QUICKI). Body mass index was calculated by weighing and measuring the heights of all study participants at the beginning of the investigation. Body fat mass and body lean mass were calculated as percentages of body weight by bioelectrical impedance analysis of body composition. Sustanon 250 (a combination of 4 testosterone) was administered intramuscularly once every 3 weeks for 6 months to male patients with IHH after a basal anthropometric, biochemical, and hormonal evaluation. The response to therapy was monitored by regular clinical examinations and serum testosterone measurements. After 6 months of testosterone treatment, the entire anthropometric, biochemical, and hormonal evaluation was repeated 14 days after the last injection of testosterone.

**RESULTS:** Before treatment, male patients with IHH had higher fasting plasma glucose concentrations, higher fasting plasma insulin levels, a higher HOMA-IR score, and a lower QUICKI when compared with the control group. After testosterone treatment in the patient group, the HOMA-IR score decreased dramatically to the level in the control group. The high body fat mass of the male patients with IHH was reduced significantly after testosterone treatment, concomitant with significant increases in body mass index and body lean mass.

**CONCLUSION:** Insulin sensitivity improves and body fat mass decreases with long-term testosterone replacement therapy.

Endocr Pract. 2007 Oct;13(6):629-35

## A DOSE-RESPONSE STUDY OF TESTOSTERONE ON SEXUAL DYSFUNCTION AND FEATURES OF THE METABOLIC SYNDROME USING TESTOSTERONE GEL AND PARENTERAL TESTOSTERONE UNDECANOATE.

The objective of this study was to observe the dose-response effects of testosterone (T) treatment on symptoms of sexual dysfunction and the metabolic syndrome. Two cohorts of elderly men with late-onset hypogonadism were followed over 9 months. Group 1, consisting of 28 men (mean age, 61 years; mean T level, 2.07 +/- 0.50 ng/mL), received long-acting T undecanoate (TU; 1000 mg); group 2, composed of 27 men (mean age, 60 years; mean T level, 2.24 +/- 0.41 ng/mL), received T gel (50 mg/day) for 9 months. In patients treated with T gel, plasma T levels rose from 2.24 +/- 0.41 to 2.95 +/- 0.52 (statistically significant) at 3 months, 3.49 +/- 0.89 (statistically significant) at 6 months, and 3.80 +/- 0.73 ng/mL at 9 months (T level at 6 months was compared with T level at 3 months). With TU, plasma T levels rose from 2.08 +/- 0.56 to 4.81 +/- 0.83 (statistically significant) at 3 months, 5.29 +/- 0.91 at 6 months, and 5.40 +/- 0.77 ng/mL at 9 months. With TU, the plasma T levels were statistically significantly higher than with T gel. With TU, there was a greater improvement in sexual symptoms and in symptoms of the metabolic syndrome. With both treatments, changes in waist circumference correlated with changes in total, low-density, and high-density lipoprotein cholesterol. Parameters of safety were not different between the 2 treatments. T administration had a beneficial effect on sexual dysfunction and symptoms of the metabolic syndrome in elderly men. The higher plasma levels of T generated with TU than with T gel were clearly more effective, indicating that there is a T dose-effect relationship.

J Androl. 2008 Jan-Feb;29(1):102-5

## BODY COMPOSITION, METABOLIC SYNDROME AND TESTOSTERONE IN AGEING MEN.

The ageing process in men is marked by changes in body composition (loss of fat-free mass (FFM) and skeletal muscle, and gain in fat mass (FM)) and is associated with a decline in serum testosterone. Correlations between these aspects of ageing and the acknowledged role of exogenous testosterone in reversing the loss of FFM and gain in FM seen in adult men with congenital or acquired hypoandrogenism have led to the hypothesis that testosterone therapy in ageing men will result in favourable changes in body composition and may improve metabolic status and/or cardiovascular risk. Data from randomized controlled trials of testosterone therapy in ageing men addressing the endpoints of body composition and components of the metabolic syndrome and cardiovascular risk factors are reviewed, and the impact of the increasing prevalence of obesity on these relationships is considered.

Int J Impot Res. 2007 Sep-Oct;19(5):448-57

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