

JOURNAL  
ABSTRACTS

## Pyridoxamine

**PYRIDOXAMINE, AN INHIBITOR OF ADVANCED GLYCATION AND LIPOXIDATION REACTIONS: A NOVEL THERAPY FOR TREATMENT OF DIABETIC COMPLICATIONS.**

Pyridoxamine (PM), originally described as a post-Amadori inhibitor of formation of advanced glycation end-products (AGEs), also inhibits the formation of advanced lipoxidation end-products (ALEs) on protein during lipid peroxidation reactions. In addition to inhibition of AGE/ALE formation, PM has a strong lipid-lowering effect in streptozotocin (STZ)-induced diabetic and Zucker obese rats, and protects against the development of nephropathy in both animal models. PM also inhibits the development of retinopathy and neuropathy in the STZ-diabetic rat. Several products of reaction of PM with intermediates in lipid autoxidation have been identified in model reactions in vitro and in the urine of diabetic and obese rats, confirming the action of PM as an AGE/ALE inhibitor. PM appears to act by a mechanism analogous to that of AGE-breakers, by reaction with dicarbonyl intermediates in AGE/ALE formation. This review summarizes current knowledge on the mechanism of formation of AGE/ALEs, proposes a mechanism of action of PM, and summarizes the results of animal model studies on the use of PM for inhibiting AGE/ALE formation and development of complications of diabetes and hyperlipidemia.

Arch Biochem Biophys. 2003 Nov 1;419(1):41-9

**A POST-AMADORI INHIBITOR PYRIDOXAMINE ALSO INHIBITS CHEMICAL MODIFICATION OF PROTEINS BY SCAVENGING CARBONYL INTERMEDIATES OF CARBOHYDRATE AND LIPID DEGRADATION.**

Reactive carbonyl compounds are formed during autoxidation of carbohydrates and peroxidation of lipids. These compounds are intermediates in the formation of advanced glycation end products (AGE) and advanced lipoxidation end products (ALE) in tissue proteins during aging and in chronic disease. We studied the reaction of carbonyl compounds glyoxal (GO) and glycolaldehyde (GLA) with pyridoxamine (PM), a potent post-Amadori inhibitor of AGE formation in vitro and of development of renal and retinal pathology in diabetic animals. PM reacted rapidly with GO and GLA in neutral, aqueous buffer, forming a Schiff base intermediate that cyclized to a hemiaminal adduct by intramolecular reaction with the phenolic hydroxyl group of PM. This bicyclic intermediate dimerized to form a five-ring compound with a central piperazine ring, which was characterized by electrospray ionization-liquid chromatography/mass spectrometry, NMR, and x-ray crystallography. PM also inhibited the modification of lysine residues and loss of enzymatic activity of RNase in the presence of GO and GLA and inhibited formation of the AGE/ALE N(epsilon)-(carboxymethyl)lysine during reaction of GO and GLA with bovine serum albumin. Our data suggest that the AGE/ALE inhibitory activity and the therapeutic effects of PM observed in diabetic animal models depend, at least in part, on its ability to trap reactive carbonyl intermediates in AGE/ALE formation, thereby inhibiting the chemical modification of tissue proteins.

J Biol Chem. 2002 Feb 1;277(5):3397-403

**PROPAGATION OF PROTEIN GLYCATION DAMAGE INVOLVES MODIFICATION OF TRYPTOPHAN RESIDUES VIA REACTIVE OXYGEN SPECIES: INHIBITION BY PYRIDOXAMINE.**

Nonenzymatic modification of proteins is one of the key pathogenic factors in diabetic complications. Uncovering the mechanisms of protein damage caused by glucose is fundamental to understanding this pathogenesis and in the development of new therapies. We investigated whether the mechanism involving reactive oxygen species can propagate protein damage in glycation reactions beyond the classical modifications of lysine and arginine residues. We have demonstrated that glucose can cause specific oxidative modification of tryptophan residues in lysozyme and inhibit lysozyme activity. Furthermore, modification of tryptophan residues was also induced by purified albumin-Amadori, a ribose-derived model glycation intermediate. The AGE inhibitor pyridoxamine (PM) prevented the tryptophan modification, whereas another AGE inhibitor and strong carbonyl scavenger, aminoguanidine, was ineffective. PM specifically inhibited generation of hydroxyl radical from albumin-Amadori and protected tryptophan from oxidation by hydroxyl radical species. We conclude that oxidative degradation of either glucose or the protein-Amadori intermediate causes oxidative modification of protein tryptophan residues via hydroxyl radical and can affect protein function under physiologically relevant conditions. This oxidative stress-induced structural and functional protein damage can be ameliorated by PM via sequestration of catalytic metal ions and scavenging of hydroxyl radical, a mechanism that may

contribute to the reported therapeutic effects of PM in the complications of diabetes.

Free Radic Biol Med. 2008 Apr 1;44(7):1276-85

### **THIAMINE PYROPHOSPHATE AND PYRIDOXAMINE INHIBIT THE FORMATION OF ANTIGENIC ADVANCED GLYCATION END-PRODUCTS: COMPARISON WITH AMINO GUANIDINE.**

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

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

### **PYRIDOXAMINE PROTECTS PROTEINS FROM FUNCTIONAL DAMAGE BY 3-DEOXYGLUCOSONE: MECHANISM OF ACTION OF PYRIDOXAMINE.**

Pyridoxamine (PM) is a promising drug candidate for treatment of diabetic nephropathy. The therapeutic effect of PM has been demonstrated in multiple animal models of diabetes and in phase II clinical trials. However, the mechanism of PM therapeutic action is poorly understood. One potential mechanism is scavenging of pathogenic reactive carbonyl species (RCS) found to be elevated in diabetes. We have suggested previously that the pathogenicity of RCS methylglyoxal (MGO) may be due to modification of critical arginine residues in matrix proteins and interference with renal cell-matrix interactions. We have also shown that this MGO effect can be inhibited by PM (Pedchenko et al. (2005) Diabetes 54, 2952-2960). These findings raised the questions of whether the effect is specific to MGO, whether other structurally different physiological RCS can act via the same mechanism, and whether their action is amenable to PM protection. In the present study, we have shown that the important physiological RCS 3-deoxyglucosone (3-DG) can damage protein functionality, including the ability of collagen IV to interact with glomerular mesangial cells. We have also demonstrated that PM can protect against 3-DG-induced protein damage via a novel mechanism that includes transient adduction of 3-DG by PM followed by irreversible PM-mediated oxidative cleavage of 3-DG. Our results suggest that, in diabetic nephropathy, the therapeutic effect of PM is achieved, in part, via protection of renal cell-matrix interactions from damage by a variety of RCS. Our data emphasize the potential importance of the contribution by 3-DG, along with other more reactive RCS, to this pathogenic mechanism.

Biochemistry. 2008 Jan 22;47(3):997-1006

### **PYRIDOXINE AND PYRIDOXAMINE INHIBITS SUPEROXIDE RADICALS AND PREVENTS LIPID PEROXIDATION, PROTEIN GLYCOSYLATION, AND (NA<sup>+</sup> + K<sup>+</sup>)-ATPASE ACTIVITY REDUCTION IN HIGH GLUCOSE-TREATED HUMAN ERYTHROCYTES.**

Vitamin B(6) (pyridoxine) supplementation has been found beneficial in preventing diabetic neuropathy and retinopathy, and the glycosylation of proteins. Oxygen radicals and oxidative damage have been implicated in the cellular dysfunction and complications of diabetes. This study was undertaken to test the hypothesis that pyridoxine (P) and pyridoxamine (PM) inhibit superoxide radical production, reduce lipid peroxidation and glycosylation, and increase the (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity in high glucose-exposed red blood cells (RBC). Superoxide radical production was assessed by the reduction of cytochrome C by glucose in the presence and absence of P or PM in a cell-free buffered solution. To examine cellular effects, washed normal human RBC were treated with control and high glucose concentrations with and without P or PM. Both P and PM significantly lowered lipid peroxidation and glycated hemoglobin (HbA(1)) formation in high glucose-exposed RBC. P and PM significantly prevented the reduction in (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity in high glucose-treated RBC. Thus, P or PM can inhibit oxygen radical production, which in turn prevents the lipid peroxidation, protein glycosylation, and (Na<sup>+</sup> + K<sup>+</sup>)-ATPase activity reduction induced by the hyperglycemia. This study describes a new biochemical mechanism by which P or PM supplementation may delay or inhibit the development of complications in diabetes.

Free Radic Biol Med. 2001 Feb 1;30(3):232-7

### **THE AGE INHIBITOR PYRIDOXAMINE INHIBITS LIPEMIA AND DEVELOPMENT OF RENAL AND VASCULAR DISEASE IN ZUCKER OBESE RATS.**

**BACKGROUND:** In previous studies, pyridoxamine (PM) limited the formation of advanced glycation end products (AGEs) and development of nephropathy in streptozotocin-diabetic rats without affecting glycemic control. However, the lipid-lowering effects of PM and the correlation of plasma cholesterol and triglycerides with AGEs in skin collagen suggested that lipids might be an

important source of AGEs in the diabetic rat. This study addresses the effects of hyperlipidemia on formation of advanced glycation and lipoxidation end products (AGE/ALEs) and the effects of PM on hyperlipidemia, hypertension, AGE/ALE formation, and development of nephropathy in the nondiabetic, Zucker obese rat. METHODS: Three groups of Zucker rats were studied: lean (Fa/fa), untreated fatty (fa/fa), and fa/fa treated with PM (2 g/L drinking water). Blood pressure, plasma lipids and creatinine, and urinary albumin were measured monthly. AGE/ALEs were measured in skin collagen by high-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS). Changes in wall thickness of the aorta and renal arterioles were evaluated by light microscopy. RESULTS: AGE/ALEs formation was increased two- to threefold in skin collagen of obese versus lean rats. PM inhibited the increases in AGE/ALEs in collagen, and significantly decreased the rise in plasma triglycerides, cholesterol, and creatinine, corrected hypertension and thickening of the vascular wall, and nearly normalized urinary protein and albumin excretion in Zucker fa/fa rats. CONCLUSION: Lipids are an important source of chemical modification of tissue proteins, even in the absence of hyperglycemia. PM inhibited AGE/ALE formation and hyperlipidemia and protected against renal and vascular pathology in a nondiabetic model.

Kidney Int. 2003 Jun;63(6):2123-33

### **PYRIDOXAMINE LOWERS KIDNEY CRYSTALS IN EXPERIMENTAL HYPEROXALURIA: A POTENTIAL THERAPY FOR PRIMARY HYPEROXALURIA.**

**BACKGROUND:** Primary hyperoxaluria is a rare genetic disorder of glyoxylate metabolism that results in overproduction of oxalate. The disease is characterized by severe calcium oxalate nephrolithiasis and nephrocalcinosis, resulting in end-stage renal disease (ESRD) early in life. Most patients eventually require dialysis and kidney transplantation, usually in combination with the replacement of the liver. Reduction of urinary oxalate levels can efficiently decrease calcium oxalate depositions; yet, no treatment is available that targets oxalate biosynthesis. In previous in vitro studies, we demonstrated that pyridoxamine can trap reactive carbonyl compounds, including intermediates of oxalate biosynthesis. **METHODS:** The effect of PM on urinary oxalate excretion and kidney crystal formation was determined using the ethylene glycol rat model of hyperoxaluria. Animals were given 0.75% to 0.8% ethylene glycol in drinking water to establish and maintain hyperoxaluria. After 2 weeks, pyridoxamine treatment (180 mg/day/kg body weight) started and continued for an additional 2 weeks. Urinary creatinine, glycolate, oxalate, and calcium were measured along with the microscopic analysis of kidney tissues for the presence of calcium oxalate crystals. **RESULTS:** Pyridoxamine treatment resulted in significantly lower (by approximately 50%) levels of urinary glycolate and oxalate excretion compared to untreated hyperoxaluric animals. This was accompanied by a significant reduction in calcium oxalate crystal formation in papillary and medullary areas of the kidney. **CONCLUSION:** These results, coupled with favorable toxicity profiles of pyridoxamine in humans, show promise for therapeutic use of pyridoxamine in primary hyperoxaluria and other kidney stone diseases.

Kidney Int. 2005 Jan;67(1):53-60

### **RENOPROTECTIVE EFFECTS OF THE AGE-INHIBITOR PYRIDOXAMINE IN EXPERIMENTAL CHRONIC ALLOGRAFT NEPHROPATHY IN RATS.**

**BACKGROUND:** Advanced glycation end products (AGEs) are involved in diabetic nephropathy (DN). The AGE formation inhibitor pyridoxamine (PM) is renoprotective in DN and in normoglycaemic obese Zucker rats. In chronic allograft nephropathy (CAN), renal AGE accumulation occurs as well. **METHODS:** To investigate whether inhibition of AGE formation is renoprotective in CAN, we studied the Fisher 344 to Lewis (F-L) allograft rat model of experimental CAN. Fisher to Fisher (F-F) isografts served as controls. Proteinuria, renal function and renal histology of untreated transplanted rats (F-L n = 8, F-F n = 8) were compared to rats receiving PM 2 g/l in drinking water for 20 weeks starting at transplantation (F-L n = 5, F-F n = 10). All rats received cyclosporin A (1.5 mg/kg/day) for 10 days after transplantation to prevent early acute rejection. **RESULTS:** Compared to untreated allografts, PM significantly decreased proteinuria (76 +/- 18 vs 29 +/- 3 mg/day), serum creatinine (130 +/- 12 vs 98 +/- 5 micromol/l), focal glomerulosclerosis (116 +/- 27 vs 16 +/- 5 AU), glomerular macrophage influx (5.6 +/- 0.6 vs 3.3 +/- 1.0), interstitial fibrosis (132 +/- 24 vs 76 +/- 2 AU) and interstitial macrophage influx (47.0 +/- 8.7 vs 15.4 +/- 5.0). Moreover, PM significantly ameliorated tubular accumulation of pentosidine, compared to untreated allografts (2.5 +/- 0.6 vs 0.3 +/- 0.3, all p < 0.05). In the isograft controls, these values did not differ between untreated and PM treated rats. **CONCLUSION:** PM exerts renoprotective effects and decreases renal pentosidine accumulation in experimental CAN, suggesting a detrimental role for renal AGE accumulation in the pathogenesis of renal damage in this non-diabetic model. These results indicate that inhibition of AGE formation might be a useful adjunct therapy to attenuate CAN.

Nephrol Dial Transplant. 2008 Feb;23(2):518-24

### **INHIBITION OF ADVANCED GLYCATION AND ABSENCE OF GALECTIN-3 PREVENT BLOOD-RETINAL BARRIER DYSFUNCTION DURING SHORT-TERM DIABETES.**

Breakdown of the inner blood-retinal barrier (iBRB) occurs early in diabetes and is central to the development of sight-threatening diabetic macular edema (DME) as retinopathy progresses. In the current study, we examined how advanced glycation end

products (AGEs) forming early in diabetes could modulate vasopermeability factor expression in the diabetic retina and alter inter-endothelial cell tight junction (TJ) integrity leading to iBRB dysfunction. We also investigated the potential for an AGE inhibitor to prevent this acute pathology and examined a role of the AGE-binding protein galectin-3 (Gal-3) in AGE-mediated cell retinal pathophysiology. Diabetes was induced in C57/BL6 wild-type (WT) mice and in Gal-3(-/-) transgenic mice. Blood glucose was monitored and AGE levels were quantified by ELISA and immunohistochemistry. The diabetic groups were subdivided, and one group was treated with the AGE-inhibitor pyridoxamine (PM) while separate groups of WT and Gal-3(-/-) mice were maintained as nondiabetic controls. iBRB integrity was assessed by Evans blue assay alongside visualisation of TJ protein complexes via occludin-1 immunolocalization in retinal flat mounts. Retinal expression levels of the vasopermeability factor VEGF were quantified using real-time RT-PCR and ELISA. WT diabetic mice showed significant AGE-immunoreactivity in the retinal microvasculature and also showed significant iBRB breakdown ( $P < .005$ ). These diabetics had higher VEGF mRNA and protein expression in comparison to controls ( $P < .01$ ). PM-treated diabetics had normal iBRB function and significantly reduced diabetes-mediated VEGF expression. Diabetic retinal vessels showed disrupted TJ integrity when compared to controls, while PM-treated diabetics demonstrated near-normal configuration. Gal-3(-/-) mice showed significantly less diabetes-mediated iBRB dysfunction, junctional disruption, and VEGF expression changes than their WT counterparts. The data suggests an AGE-mediated disruption of iBRB via upregulation of VEGF in the diabetic retina, possibly modulating disruption of TJ integrity, even after acute diabetes. Prevention of AGE formation or genetic deletion of Gal-3 can effectively prevent these acute diabetic retinopathy changes.

Exp Diabetes Res. 2007;2007:51837

### **THE AGE INHIBITOR PYRIDOXAMINE INHIBITS DEVELOPMENT OF RETINOPATHY IN EXPERIMENTAL DIABETES.**

We examined the ability of pyridoxamine (PM), an inhibitor of formation of advanced glycation end products (AGEs) and lipoxidation end products (ALEs), to protect against diabetes-induced retinal vascular lesions. The effects of PM were compared with the antioxidants vitamin E (VE) and R-alpha-lipoic acid (LA) in streptozotocin-induced diabetic rats. Animals were given either PM (1 g/l drinking water), VE (2,000 IU/kg diet), or LA (0.05%/kg diet). After 29 weeks of diabetes, retinas were examined for pathogenic changes, alterations in extracellular matrix (ECM) gene expression, and accumulation of the immunoreactive AGE/ALE N(epsilon)-(carboxymethyl)lysine (CML). Acellular capillaries were increased more than threefold, accompanied by significant upregulation of laminin immunoreactivity in the retinal microvasculature. Diabetes also increased mRNA expression for fibronectin (2-fold), collagen IV (1.6-fold), and laminin beta chain (2.6-fold) in untreated diabetic rats compared with nondiabetic rats. PM treatment protected against capillary drop-out and limited laminin protein upregulation and ECM mRNA expression and the increase in CML in the retinal vasculature. VE and LA failed to protect against retinal capillary closure and had inconsistent effects on diabetes-related upregulation of ECM mRNAs. These results indicate that the AGE/ALE inhibitor PM protected against a range of pathological changes in the diabetic retina and may be useful for treating diabetic retinopathy.

Diabetes. 2002 Sep;51(9):2826-32

### **EFFECTS OF PYRIDOXAMINE IN COMBINED PHASE 2 STUDIES OF PATIENTS WITH TYPE 1 AND TYPE 2 DIABETES AND OVERT NEPHROPATHY.**

**BACKGROUND/AIMS:** Treatments of diabetic nephropathy (DN) delay the onset of end-stage renal disease. We report the results of safety/tolerability studies in patients with overt nephropathy and type 1/type 2 diabetes treated with pyridoxamine, a broad inhibitor of advanced glycation. **METHODS:** The two 24-week studies were multicenter Phase 2 trials in patients under standard-of-care. In PYR-206, patients were randomized 1:1 and had baseline serum creatinine (bSCr)  $\leq 2.0$  mg/dl. In PYR-205/207, randomization was 2:1 and bSCr was  $\leq 2.0$  for PYR-205 and  $> 2.0$  but  $\leq 3.5$  mg/dl for PYR-207. Treated patients (122 active, 90 placebo) received 50 mg pyridoxamine twice daily in PYR-206; PYR-205/207 patients were escalated to 250 mg twice daily. **RESULTS:** Adverse events were balanced between the groups ( $p = \text{NS}$ ). Slight imbalances, mainly in the PYR-205/207 groups, were noted in deaths (from diverse causes,  $p = \text{NS}$ ) and serious adverse events ( $p = 0.05$ ) that were attributed to pre-existing conditions. In a merged data set, pyridoxamine significantly reduced the change from baseline in serum creatinine ( $p < 0.03$ ). In patients similar to the RENAAL/IDNT studies (bSCr  $> 1.3$  mg/dl, type 2 diabetes), a treatment effect was observed on the rise in serum creatinine ( $p = 0.007$ ). No differences in urinary albumin excretion were seen. Urinary TGF-beta1 also tended to decrease with pyridoxamine ( $p = 0.049$ ) as did the CML and CEL AGEs. **CONCLUSION:** These data provide a foundation for further evaluation of this AGE inhibitor in DN.

Am J Nephrol. 2007;27(6):605-14

### **PYRIDOXAMINE, AN INHIBITOR OF ADVANCED GLYCATION REACTIONS, ALSO INHIBITS ADVANCED LIPOXIDATION REACTIONS. MECHANISM OF ACTION OF PYRIDOXAMINE.**

Maillard or browning reactions lead to formation of advanced glycation end products (AGEs) on protein and contribute to the increase in chemical modification of proteins during aging and in diabetes. AGE inhibitors such as aminoguanidine and

pyridoxamine (PM) have proven effective in animal model and clinical studies as inhibitors of AGE formation and development of diabetic complications. We report here that PM also inhibits the chemical modification of proteins during lipid peroxidation (lipoxidation) reactions in vitro, and we show that it traps reactive intermediates formed during lipid peroxidation. In reactions of arachidonate with the model protein RNase, PM prevented modification of lysine residues and formation of the advanced lipoxidation end products (ALEs) N(epsilon)-(carboxymethyl)lysine, N(epsilon)-(carboxyethyl)lysine, malondialdehyde-lysine, and 4-hydroxynonenal-lysine. PM also inhibited lysine modification and formation of ALEs during copper-catalyzed oxidation of low density lipoprotein. Hexanoic acid amide and nonanedioic acid monoamide derivatives of PM were identified as major products formed during oxidation of linoleic acid in the presence of PM. We propose a mechanism for formation of these products from the 9- and 13-oxo-decadienoic acid intermediates formed during peroxidation of linoleic acid. PM, as a potent inhibitor of both AGE and ALE formation, may prove useful for limiting the increased chemical modification of tissue proteins and associated pathology in aging and chronic diseases, including both diabetes and atherosclerosis.

J Biol Chem. 2000 Jul 14;275(28):21177-84

### **LASER ACUPUNCTURE: PAST, PRESENT, AND FUTURE.**

Laser acupuncture is defined as the stimulation of traditional acupuncture points with low-intensity, nonthermal laser irradiation. Although the therapeutic use of laser acupuncture is rapidly gaining in popularity, objective evaluation of its efficacy in published studies is difficult because treatment parameters such as wavelength, irradiance, and beam profile are seldom fully described. The depth of laser energy transmission, likely an important determinant of efficacy, is governed not only by these parameters, but also by skin properties such as thickness, age, and pigmentation-factors which have also received little consideration in laser acupuncture. Despite the frequently equivocal nature of the published laser studies, recent evidence of visual cortex activation by laser acupuncture of foot points, together with the known ability of laser irradiation to induce cellular effects at subthermal thresholds, provides impetus for further research.

Lasers Med Sci. 2004;19(2):69-80

### **LOW LEVEL LASER THERAPY FOR NONSPECIFIC LOW-BACK PAIN.**

**BACKGROUND:** Low-back pain (LBP) is a major health problem and a major cause of medical expenses and disablement. Low level laser therapy (LLLT) can be used to treat musculoskeletal disorders such as back pain. **OBJECTIVES:** To assess the effects of LLLT in patients with non-specific LBP. **SEARCH STRATEGY:** We searched CENTRAL (The Cochrane Library 2005, Issue 2), MEDLINE, CINAHL, EMBASE, AMED and PEDro from their start to November 2007 with no language restrictions. We screened references in the included studies and in reviews and conducted citation tracking of identified RCTs and reviews using Science Citation Index. We also contacted content experts. **SELECTION CRITERIA:** Randomised controlled clinical trials (RCTs) investigating LLLT to treat non-specific low-back pain were included. **DATA COLLECTION AND ANALYSIS:** Two authors independently assessed methodological quality using the criteria recommended by the Cochrane Back Review Group and extracted data. Studies were qualitatively and quantitatively analysed according to Cochrane Back Review Group guideline. **MAIN RESULTS:** Seven heterogeneous English language RCTs with reasonable quality were included. Three small studies (168 people) separately showed statistically significant but clinically unimportant pain relief for LLLT versus sham therapy for sub-acute and chronic low-back pain at short-term and intermediate-term follow-up (up to six months). One study (56 people) showed that LLLT was more effective than sham at reducing disability in the short term. Three studies (102 people) reported that LLLT plus exercise were not better than exercise, with or without sham in the short-term in reducing pain or disability. Two studies (90 people) reported that LLLT was not more effective than exercise, with or without sham in reducing pain or disability in the short term. Two small trials (151 people) independently found that the relapse rate in the LLLT group was significantly lower than in the control group at the six-month follow-up. No side effects were reported. **AUTHORS' CONCLUSIONS:** Based on the heterogeneity of the populations, interventions and comparison groups, we conclude that there are insufficient data to draw firm conclusions on the clinical effect of LLLT for low-back pain. There is a need for further methodologically rigorous RCTs to evaluate the effects of LLLT compared to other treatments, different lengths of treatment, wavelengths and dosages.

Cochrane Database Syst Rev. 2008 Apr 16;(2):CD005107

### **A SYSTEMATIC REVIEW WITH PROCEDURAL ASSESSMENTS AND META-ANALYSIS OF LOW LEVEL LASER THERAPY IN LATERAL ELBOW TENDINOPATHY (TENNIS ELBOW).**

**BACKGROUND:** Recent reviews have indicated that low level level laser therapy (LLLT) is ineffective in lateral elbow tendinopathy (LET) without assessing validity of treatment procedures and doses or the influence of prior steroid injections. **METHODS:** Systematic review with meta-analysis, with primary outcome measures of pain relief and/or global improvement and subgroup analyses of methodological quality, wavelengths and treatment procedures. **RESULTS:** 18 randomised placebo-controlled trials (RCTs) were identified with 13 RCTs (730 patients) meeting the criteria for meta-analysis. 12 RCTs satisfied half or more of the methodological criteria. Publication bias was detected by Egger's graphical test, which showed a negative direction of bias. Ten of the trials included patients with poor prognosis caused by failed steroid injections or other treatment failures, or long symptom duration or severe baseline pain. The weighted mean difference (WMD) for pain relief was 10.2 mm [95% CI: 3.0 to 17.5] and the RR for global improvement was 1.36 [1.16 to 1.60]. Trials which targeted acupuncture points reported negative results, as did trials with wavelengths 820, 830, and 1064 nm. In a subgroup of five trials with 904 nm lasers and one trial with 632 nm wavelength where the lateral elbow tendon insertions were directly irradiated, WMD for pain relief was 17.2 mm [95% CI: 8.5 to 25.9] and 14.0 mm [95% CI: 7.4 to 20.6] respectively, while RR for global pain improvement was only reported for 904 nm at 1.53

[95% CI: 1.28 to 1.83]. LLLT doses in this subgroup ranged between 0.5 and 7.2 Joules. Secondary outcome measures of painfree grip strength, pain pressure threshold, sick leave and follow-up data from 3 to 8 weeks after the end of treatment, showed consistently significant results in favour of the same LLLT subgroup ( $p < 0.02$ ). No serious side-effects were reported. **CONCLUSION:** LLLT administered with optimal doses of 904 nm and possibly 632 nm wavelengths directly to the lateral elbow tendon insertions, seem to offer short-term pain relief and less disability in LET, both alone and in conjunction with an exercise regimen. This finding contradicts the conclusions of previous reviews which failed to assess treatment procedures, wavelengths and optimal doses.

BMC Musculoskelet Disord. 2008 May 29;9:75

### **EVALUATION OF LOW-LEVEL LASER THERAPY IN THE TREATMENT OF TEMPOROMANDIBULAR DISORDERS.**

**OBJECTIVE:** The purpose of this study was to assess the effectiveness of low-level laser therapy (LLL) in the treatment of myogenic originated temporomandibular disorders (TMD). **BACKGROUND DATA:** Limited studies have demonstrated that LLLT may have a therapeutic effect on the treatment of TMD. **METHODS:** Thirty-nine patients with myogenic TMD-associated orofacial pain, limited mandibular movements, chewing difficulties, and tender points were included in this study. Twenty-four of them were treated with LLLT for 10 sessions per day excluding weekends as test group, and 15 patients with the same protocol received placebo laser treatment as a control group. These parameters were assessed just before, just after, and 1 month after the treatment. **RESULTS:** Maximal mouth-opening improvement, and reductions in pain and chewing difficulty were statistically significant in the test group when compared with the control group. Statistically significant improvements were also detected between two groups regarding reduction in the number of tender points. **CONCLUSION:** Based on the results of this placebo-controlled report, LLLT is an appropriate treatment for TMD and should be considered as an alternative to other methods.

Photomed Laser Surg. 2006 Oct;24(5):637-41

### **EFFECT OF LOW LEVEL LASER THERAPY IN RHEUMATOID ARTHRITIS PATIENTS WITH CARPAL TUNNEL SYNDROME.**

**OBJECTIVE:** the aim of the present study was to evaluate the efficacy of low level laser therapy (LLL) in patients with rheumatoid arthritis (RA) with carpal tunnel syndrome (CTS). **MATERIAL AND METHODS:** a total of 19 patients with the diagnosis of CTS in 19 hands were included and randomly assigned to two treatment groups; LLLT (Group 1) (10 hands) with dosage 1.5 J/ per point and placebo laser therapy group (Group 2) (9 hands). A Gallium-Aluminum-Arsenide diode laser device was used as a source of low power laser with a power output of 50 mW and wavelength of 780 nm. All treatments were applied once a day on week days for a total period of 10 days. Clinical assessments were performed at baseline, at the end of the treatment and at month 3. Tinel and Phalen signs were tested in all patients. Patients were evaluated for such clinical parameters as functional status scale (FSS), visual analogue scale (VAS), symptom severity scale (SSS) and grip-strength. However, electrophysiological examination was performed on all hands. Results were given with descriptive statistics and confidence intervals between group means at 3 months adjusted for outcome at baseline and for the difference between unadjusted group proportions. **RESULTS:** clinical and electrophysiological parameters were similar at baseline in both groups. Improvements were significantly more pronounced in the LLLT group than placebo group. A comparison between groups showed significant improvements in pain score and functional status scale score. Group mean differences at 3 months adjusted at baseline were found to be statistically significant for pain score and functional status scale score. The 95% significant confidence intervals were [-15 - (-5)] and [-5 - (-2)] respectively. There were no statistically significant differences in other clinical and electrophysiological parameters between groups at 3 months. **CONCLUSIONS:** our study results indicate that LLLT and placebo laser therapy seems to be effective for pain and hand function in CTS. We, therefore, suggest that LLLT may be used as a good alternative treatment method in CTS patients with RA.

Swiss Med Wkly. 2007 Jun 16;137(23-24):347-52

### **CLINICAL EFFECT OF CO(2) LASER IN REDUCING PAIN IN ORTHODONTICS.**

**OBJECTIVE:** To test the hypothesis that there is no difference in the pain associated with orthodontic force application after the application of local CO(2) laser irradiation to the teeth involved. **MATERIALS AND METHODS:** Separation modules were placed at the distal contacts of the maxillary first molars in 90 patients in this single-blinded study. In 60 of these patients (42 females and 18 males; mean age = 19.22 years) this was immediately followed by laser therapy. The other 30 patients (18 females and 12 males; mean age = 18.8 years) did not receive active laser irradiation. Patients were then instructed to rate their levels of pain on a visual analog scale over time, and the amount of tooth movement was analyzed. **RESULTS:** Significant pain reductions were observed with laser treatment from immediately after insertion of separators through day 4, but no differences from the nonirradiated control side were noted thereafter. No significant difference was noted in the amount of tooth movement between the irradiated and nonirradiated group. **CONCLUSIONS:** The hypothesis was rejected. The results suggest that local CO(2) laser irradiation will reduce pain associated with orthodontic force application without interfering with the tooth movement.

## **SYSTEMATIC REVIEW OF THE LITERATURE OF LOW-LEVEL LASER THERAPY (LLLT) IN THE MANAGEMENT OF NECK PAIN.**

**BACKGROUND AND OBJECTIVES:** Low-level laser therapy (LLLT) is widely used in the treatment of musculoskeletal pain. However, there is controversy over its true efficacy. We aimed to determine the efficacy of LLLT in the treatment of neck pain through systematically reviewing the literature. **STUDY DESIGN/MATERIALS AND METHODS:** A search of computerized bibliographic databases covering medicine, physiotherapy, allied health, complementary medicine, and biological sciences was undertaken from date of inception until February 2004 for randomized controlled trials of LLLT for neck pain. A comprehensive list of search terms was applied and explicit inclusion criteria were developed a priori. Twenty studies were identified, five of which met the inclusion criteria. **RESULTS:** Significant positive effects were reported in four of five trials in which infrared wavelengths ( $\lambda = 780, 810-830, 904, 1,064$  nm) were used. Heterogeneity in outcome measures, results reporting, doses, and laser parameters precluded formal meta-analysis. Effect sizes could be calculated for only two of the studies. **CONCLUSIONS:** This review provides limited evidence from one RCT for the use of infrared laser for the treatment of acute neck pain ( $n = 71$ ) and chronic neck pain from four RCTs ( $n = 202$ ). Larger studies are required to confirm the positive findings and determine the most effective laser parameters, sites and modes of application.

Lasers Surg Med. 2005 Jul;37(1):46-52

## **IMPORT OF RADIATION PHENOMENA OF ELECTRONS AND THERAPEUTIC LOW-LEVEL LASER IN REGARD TO THE MITOCHONDRIAL ENERGY TRANSFER.**

**OBJECTIVE:** The authors describe a consistent theoretical model of the cellular energy transfer (respiratory chain) by taking into consideration the radiation phenomena of electrons and therapeutic low level laser. **SUMMARY BACKGROUND DATA:** Biochemical models of the cellular energy transfer regard the classical corpuscular aspect of electrons as the responsible energy carriers, thereby ignoring the wave-particle dualism of the electrons and the import of radiation energy in this process. **METHODS:** The authors show the influence of radiation phenomena on the cellular energy transfer, explaining consistently some of the intermediate steps of this complex process. **RESULTS:** Because of the inherent wave-particle dualism of the electrons, it is appropriate to regard radiation phenomena to explain the cellular energy transfer. The classical biochemical models use only the particle part of the electrons as energy carriers. The connection between energy transport by radiation and the order in structures may be understood if, for instance, structurally bound energy is released during the dissolution of structures (oxidation of foodstuffs) or is again manifested (final reduction of oxygen to water). With attention to the energy values relevant for the respiratory chain, the import of electromagnetic radiation of characteristic ranges of wavelengths on the cellular energy transfer becomes evident. Depending on its wavelength, electromagnetic radiation in the form of light can stimulate macromolecules and can initiate conformation changes in proteins or can transfer energy to electrons. Low level laser from the red and the near infrared region corresponds well with the characteristic energy and absorption levels of the relevant components of the respiratory chain. This laser stimulation vitalizes the cell by increasing the mitochondrial ATP(adenosine-tri-phosphate)-production. **CONCLUSIONS:** With regard to radiation phenomena and its enhanced electron flow in the cellular energy transfer (respiratory chain), it is possible to explain the experimentally found increase of ATP-production by means of low-level laser light on a cellular level. Intense research for this biostimulative effect is still necessary.

J Clin Laser Med Surg. 1998 Jun;16(3):159-65

## **MITOCHONDRIAL MEMBRANE POTENTIAL AFTER LOW-POWER LASER IRRADIATION.**

We used the lipophilic cationic fluorescent dye 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethyl-benzimidazol-carbocyanine iodide (JC-1) to determine mitochondrial membrane potential ( $\Delta\psi$ ) in Hep-2 cells after irradiation with low-power laser ( $\lambda=635$  nm). Through this methodology it was possible to analyze the variation on mitochondrial number and  $\Delta\psi$ , in cells irradiated for 100, 150 and 200 s with energy density of 100 mJ/cm<sup>2</sup>. Our results show that JC-1 dye allows the identification of populations with different mitochondria morphology as well as the functionality of this organelle in the cells incubated for 1, 6 and 24 h, after irradiation with low-power laser.

Lasers Med Sci. 2004;18(4):204-6

## **LOW LEVEL LASER IRRADIATION STIMULATES MITOCHONDRIAL MEMBRANE POTENTIAL AND DISPERSES SUBNUCLEAR PROMYELOCYTIC LEUKEMIA PROTEIN.**

**BACKGROUND AND OBJECTIVES:** Low level laser irradiation (LLLI) is used to promote wound healing. Molecularly it is known to stimulate mitochondrial membrane potential (MMP), cytokine secretion, and cell proliferation. This study was designed to determine the influence of LLLI on the kinetics of MMP stimulation and decay, specific cytokine gene expression, and subcellular localization of promyelocytic leukemia (PML) protein on HaCaT human keratinocytes. **STUDY DESIGN/MATERIAL AND**

**METHODS:** The cells were irradiated by a 780 nm titanium-sapphire (Ti-Sa) laser with 2 J/cm<sup>2</sup> energy density. MMP was monitored with Mitotracker, a mitochondrial voltage-sensitive fluorescent dye. Cytokine gene expression was carried out using semi-quantitative-reverse transcription polymerase chain reaction. Subcellular localization of PML protein, a cell-cycle checkpoint protein, was determined using immunofluorescent staining. **RESULTS:** The fluorescence intensity of MMP was increased immediately after the end of LLLI by 148 +/- 6% over control (P<0.001). Subsequently it decayed, reaching 51 +/- 14% of the control level (P < 0.01) within 200 minutes. This decay was characterized by an exponential curve (R = 0.96) with a lifetime of 79 +/- 36 minutes (P < 0.05). Following irradiation, the expression of interleukin-1alpha, interleukin-6, and keratinocyte growth factor (KGF) genes were transiently upregulated; but the expression of the proinflammatory gene interleukin-1beta, was suppressed. The subnuclear distribution of PML was altered from discrete domains to its dispersed form within less than 1 hour after LLLI. **CONCLUSIONS:** These changes reflect a biostimulative boost that causes a shift of the cell from a quiescent to an activated stage in the cell cycle heralding proliferation and suppression of inflammation. Further characterization of MMP kinetics may provide a quantitative basis for assessment of the effect of LLLI in the clinical setting.

Lasers Surg Med. 2004;35(5):369-76

### **CELLULAR EFFECTS OF LOW POWER LASER THERAPY CAN BE MEDIATED BY NITRIC OXIDE.**

**BACKGROUND AND OBJECTIVES:** The objective of this study was to investigate the possibility of involvement of nitric oxide (NO) into the irradiation-induced increase of cell attachment. These experiments were performed with a view to exploring the cellular mechanisms of low-power laser therapy. **STUDY DESIGN/MATERIALS AND METHODS:** A suspension of HeLa cells was irradiated with a monochromatic visible-to-near infrared radiation (600-860 nm, 52 J/m<sup>2</sup>) or with a diode laser (820 nm, 8-120 J/m<sup>2</sup>) and the number of cells attached to a glass matrix was counted after 30 minute incubation at 37 degrees C. The NO donors sodium nitroprusside (SNP), glyceryl trinitrate (GTN), or sodium nitrite (NaNO<sub>2</sub>) in the concentration range 5 x 10<sup>(-9)</sup>-5 x 10<sup>(-4)</sup>M were added to the cellular suspension before or after irradiation. The action spectra and the concentration and fluence dependencies obtained were compared and analyzed. **RESULTS:** The well-structured action spectrum for the increase of the adhesion of the cells, with maxima at 619, 657, 675, 740, 760, and 820 nm, points to the existence of a photoacceptor responsible for the enhancement of this property (supposedly cytochrome c oxidase, the terminal respiratory chain enzyme), as well as signaling pathways between the cell mitochondria, plasma membrane, and nucleus. Treating the cellular suspension with SNP (5 x 10<sup>(-5)</sup>M) before irradiation significantly modifies the action spectrum for the enhancement of the cell attachment property (band maxima at 642, 685, 700, 742, 842, and 856 nm). The action of SNP, GTN, and NaNO<sub>2</sub> added before or after irradiation depends on their concentration and radiation fluence. **CONCLUSIONS:** The NO donors added to the cellular suspension before irradiation eliminate the radiation-induced increase in the number of cells attached to the glass matrix, supposedly by way of binding NO to cytochrome c oxidase. NO added to the suspension after irradiation can also inhibit the light-induced signal downstream. Both effects of NO depend on the concentration of the NO donors added. These results indicate that NO can control the irradiation-activated reactions that increase the attachment of cells.

Lasers Surg Med. 2005 Apr;36(4):307-14

### **INCREASED EXPRESSION OF MITOCHONDRIAL BENZODIAZEPINE RECEPTORS FOLLOWING LOW-LEVEL LIGHT TREATMENT FACILITATES ENHANCED PROTOPORPHYRIN IX PRODUCTION IN GLIOMA-DERIVED CELLS IN VITRO.**

**BACKGROUND AND OBJECTIVES:** This study investigates whether low-level light treatment (LLLT) can enhance the expression of peripheral-type mitochondrial benzodiazepine receptors (PBRs) on glioma-derived tumor cells, and by doing so promote the synthesis of protoporphyrin IX (PpIX) and increase the photodynamic therapy (PDT)-induced cell kill using 5-aminolevulinic acid (ALA). The endogenous photosensitizer, PpIX and related metabolites including coproporphyrin III are known to traffic into or out of the mitochondria via the PBRs situated on the outer mitochondrial membrane. Cells of astrocytic derivation within the brain express PBRs, while neurons express the central-type of benzodiazepine receptor. **STUDY DESIGN:** Astrocytoma-derived CNS-1 cells were exposed to a range of differing low-level light protocols immediately prior to PDT. LLLT involved using broad-spectrum red light of 600-800 nm or monochromatic laser light specific to 635 or 905 nm wavelength. Cells (5 x 10<sup>(5)</sup>) were exposed to a range of LLLT doses (0, 1, or 5 J/cm<sup>2</sup>) using a fixed intensity of 10 mW/cm<sup>2</sup> and subsequently harvested for cell viability, immunofluorescence, or Western blot analysis of PBR expression. The amount of PpIX within the cells was determined using chemical extraction techniques. **RESULTS:** Results confirm the induction of PBR following LLLT is dependent on the dose and wavelength of light used. Broad-spectrum red light provided the greatest cell kill following PDT, although LLLT with 635 nm or 905 nm also increased cell kill as compared to PDT alone. All LLLT regimens increased PBR expression compared to controls with corresponding increases in PpIX production. **CONCLUSIONS:** These data suggest that by selectively increasing PBR expression in tumor cells, LLLT facilitates enhanced tumor cell kill using ALA-PDT. This may further improve the selectivity and efficacy of PDT treatment of brain tumors.

Lasers Surg Med. 2007 Sep;39(8):678-84

### **830 NM LASER IRRADIATION INDUCES VARICOSITY FORMATION, REDUCES MITOCHONDRIAL MEMBRANE**

## **POTENTIAL AND BLOCKS FAST AXONAL FLOW IN SMALL AND MEDIUM DIAMETER RAT DORSAL ROOT GANGLION NEURONS: IMPLICATIONS FOR THE ANALGESIC EFFECTS OF 830 NM LASER.**

We report the formation of 830 nm (cw) laser-induced, reversible axonal varicosities, using immunostaining with beta-tubulin, in small and medium diameter, TRPV-1 positive, cultured rat DRG neurons. Laser also induced a progressive and statistically significant decrease ( $p < 0.005$ ) in MMP in mitochondria in and between static axonal varicosities. In cell bodies of the neuron, the decrease in MMP was also statistically significant ( $p < 0.05$ ), but the decrease occurred more slowly. Importantly we also report for the first time that 830 nm (cw) laser blocked fast axonal flow, imaged in real time using confocal laser microscopy and JC-1 as mitotracker. Control neurons in parallel cultures remained unaffected with no varicosity formation and no change in MMP. Mitochondrial movement was continuous and measured along the axons at a rate of 0.8 microm/s (range 0.5-2 microm/s), consistent with fast axonal flow. Photoacceptors in the mitochondrial membrane absorb laser and mediate the transduction of laser energy into electrochemical changes, initiating a secondary cascade of intracellular events. In neurons, this results in a decrease in MMP with a concurrent decrease in available ATP required for nerve function, including maintenance of microtubules and molecular motors, dyneins and kinesins, responsible for fast axonal flow. Laser-induced neural blockade is a consequence of such changes and provide a mechanism for a neural basis of laser-induced pain relief. The repeated application of laser in a clinical setting modulates nociception and reduces pain. The application of laser therapy for chronic pain may provide a non-drug alternative for the management of chronic pain.

J Peripher Nerv Syst. 2007 Mar;12(1):28-39

## Endothelial Function

### **ENDOTHELIAL FUNCTION/ENDOTHELIAL DYSFUNCTION: FROM MOLECULAR MECHANISMS TO MEASUREMENT, CLINICAL IMPLICATIONS, AND THERAPEUTIC OPPORTUNITIES.**

Endothelial dysfunction has been implicated as a key factor in the development of a wide range of cardiovascular diseases, but its definition and mechanisms vary greatly between different disease processes. This review combines evidence from cell-culture experiments, in vitro and in vivo animal models, and clinical studies to identify the variety of mechanisms involved in endothelial dysfunction in its broadest sense. Several prominent disease states, including hypertension, heart failure, and atherosclerosis, are used to illustrate the different manifestations of endothelial dysfunction and to establish its clinical implications in the context of the range of mechanisms involved in its development. The size of the literature relating to this subject precludes a comprehensive survey; this review aims to cover the key elements of endothelial dysfunction in cardiovascular disease and to highlight the importance of the process across many different conditions.

Antioxid Redox Signal. 2008 Sep;10(9):1631-74

### **IMPAIRED ENDOTHELIAL AND SMOOTH MUSCLE FUNCTIONS AND ARTERIAL STIFFNESS APPEAR BEFORE PUBERTY IN OBESE CHILDREN AND ARE ASSOCIATED WITH ELEVATED AMBULATORY BLOOD PRESSURE.**

**AIMS:** To determine whether impaired brachial endothelial (flow-mediated dilation, FMD) and smooth muscle function (nitroglycerin-mediated dilation, NTGMD), and remodelling of the common carotid artery (CCA) develop before puberty in obese children. **METHODS AND RESULTS:** Arterial intima-media thickness (IMT), FMD and NTGMD were measured by high-resolution ultrasound in 48 obese and 23 lean pre-pubertal children (8.8 +/- 1.5 years old). We assessed central pulse pressure, incremental elastic modulus (Einc), casual and ambulatory systolic (SBP) and diastolic blood pressure (DBP), and body fatness by DXA. Obese children had significantly lower FMD (4.5 +/- 4.0 vs. 8.3 +/- 1.7%), NTGMD (19.0 +/- 9.0 vs. 25.8 +/- 6.1%), and increased Einc (13.9 +/- 5.2 vs. 10.4 +/- 5.2 mmHg/10(2)), ambulatory SBP (121.3 +/- 12.6 vs. 106.6 +/- 7.1, mmHg), and DBP (69.1 +/- 5.7 vs. 63.7 +/- 4.5) than lean subjects, whereas IMT was not augmented. Ambulatory systolic hypertension was present in 47% of obese subjects. FMD, NTGMD, and Einc were correlated with body fatness, body mass index, and blood pressure (BP). **CONCLUSION:** Impaired endothelial and smooth muscle functions and altered wall material develop before puberty in obese children, however remodelling of the CCA is not yet present. Arterial dysfunction may be considered as the first marker of atherosclerosis and is associated with elevated BP. Ambulatory blood pressure monitoring may be a potential tool to improve risk stratification in obese children.

Eur Heart J. 2008 Mar;29(6):792-9

### **PHENOTYPIC HETEROGENEITY OF THE ENDOTHELIUM: I. STRUCTURE, FUNCTION, AND MECHANISMS.**

Endothelial cells, which form the inner cellular lining of blood vessels and lymphatics, display remarkable heterogeneity in structure and function. This is the first of a 2-part review focused on phenotypic heterogeneity of blood vessel endothelium. This review provides an historical perspective of our understanding of endothelial heterogeneity, discusses the scope of phenotypic diversity across the vascular tree, and addresses proximate and evolutionary mechanisms of endothelial cell heterogeneity. The overall goal is to underscore the importance of phenotypic heterogeneity as a core property of the endothelium.

Circ Res. 2007 Feb 2;100(2):158-73

### **PATHOPHYSIOLOGY, DIAGNOSIS AND PROGNOSTIC IMPLICATIONS OF ENDOTHELIAL DYSFUNCTION.**

Endothelial dysfunction (ED) in the setting of cardiovascular risk factors such as hypercholesterolemia, hypertension, diabetes mellitus, chronic smoking as well as in patients with heart failure has been shown to be at least in part dependent on the production of reactive oxygen species (ROS) such as superoxide and the subsequent decrease in vascular bioavailability of nitric oxide (NO). Methods to quantify endothelial dysfunction include forearm plethysmography, flow-dependent dilation of the brachial artery, finger-pulse plethysmography, pulse curve analysis, and quantitative coronary angiography after intracoronary administration of the endothelium-dependent vasodilator acetylcholine. Superoxide sources include the NADPH oxidase, xanthine oxidase, and mitochondria. Superoxide produced by the NADPH oxidase may react with NO released by the endothelial nitric oxide synthase (eNOS) thereby generating peroxynitrite (ONOO<sup>-</sup>), leading to eNOS uncoupling and therefore eNOS-

mediated superoxide production. The present review will discuss current concepts of how to assess endothelial function, prognostic implications of ED, mechanisms underlying ED with focus on oxidative stress and circulating biomarkers, which have been proposed to indicate endothelial dysfunction and/or damage, respectively.

Ann Med. 2008;40(3):180-96

### **SUSTAINED BENEFITS IN VASCULAR FUNCTION THROUGH FLAVANOL-CONTAINING COCOA IN MEDICATED DIABETIC PATIENTS A DOUBLE-MASKED, RANDOMIZED, CONTROLLED TRIAL.**

**OBJECTIVES:** Our goal was to test feasibility and efficacy of a dietary intervention based on daily intake of flavanol-containing cocoa for improving vascular function of medicated diabetic patients. **BACKGROUND:** Even in fully medicated diabetic patients, overall prognosis is unfavorable due to deteriorated cardiovascular function. Based on epidemiological data, diets rich in flavanols are associated with a reduced cardiovascular risk. **METHODS:** In a feasibility study with 10 diabetic patients, we assessed vascular function as flow-mediated dilation (FMD) of the brachial artery, plasma levels of flavanol metabolites, and tolerability after an acute, single-dose ingestion of cocoa, containing increasing concentrations of flavanols (75, 371, and 963 mg). In a subsequent efficacy study, changes in vascular function in 41 medicated diabetic patients were assessed after a 30-day, thrice-daily dietary intervention with either flavanol-rich cocoa (321 mg flavanols per dose) or a nutrient-matched control (25 mg flavanols per dose). Both studies were undertaken in a randomized, double-masked fashion. Primary and secondary outcome measures included changes in FMD and plasma flavanol metabolites, respectively. **RESULTS:** A single ingestion of flavanol-containing cocoa was dose-dependently associated with significant acute increases in circulating flavanols and FMD (at 2 h: from 3.7 +/- 0.2% to 5.5 +/- 0.4%,  $p < 0.001$ ). A 30-day, thrice-daily consumption of flavanol-containing cocoa increased baseline FMD by 30% ( $p < 0.0001$ ), while acute increases of FMD upon ingestion of flavanol-containing cocoa continued to be manifest throughout the study. Treatment was well tolerated without evidence of tachyphylaxia. Endothelium-independent responses, blood pressure, heart rate, and glycemic control were unaffected. **CONCLUSIONS:** Diets rich in flavanols reverse vascular dysfunction in diabetes, highlighting therapeutic potentials in cardiovascular disease.

J Am Coll Cardiol. 2008 Jun 3;51(22):2141-9

### **COCOA FLAVANOLS LOWER VASCULAR ARGINASE ACTIVITY IN HUMAN ENDOTHELIAL CELLS IN VITRO AND IN ERYTHROCYTES IN VIVO.**

The availability of L-arginine can be a rate-limiting factor for cellular NO production by nitric oxide synthases (NOS). Arginase competes with NOS for L-arginine as the common substrate. Increased arginase activity has been linked to low NO levels, and an inhibition of arginase activity has been reported to improve endothelium-dependent vasorelaxation. Based on the above, we hypothesized that an increase in the circulating NO pool following flavanol consumption could be correlated with decreased arginase activity. To test this hypothesis we (a) investigated the effects of (-)-epicatechin and its structurally related metabolites on endothelial arginase expression and activity in vitro; (b) evaluated the effects of dietary flavanol-rich cocoa on kidney arginase activity in vivo; and (c) assessed human erythrocyte arginase activity following flavanol-rich cocoa beverage consumption in a double-blind intervention study with cross-over design. The results demonstrate that cocoa flavanols lower arginase-2 mRNA expression and activity in HUVEC. Dietary intervention with flavanol-rich cocoa caused diminished arginase activity in rat kidney and, erythrocyte arginase activity was lowered in healthy humans following consumption of a high flavanol beverage in vivo.

Arch Biochem Biophys. 2008 Aug 15;476(2):211-5

### **ACUTE DARK CHOCOLATE AND COCOA INGESTION AND ENDOTHELIAL FUNCTION: A RANDOMIZED CONTROLLED CROSSOVER TRIAL.**

**BACKGROUND:** Studies suggest cardioprotective benefits of dark chocolate containing cocoa. **OBJECTIVE:** This study examines the acute effects of solid dark chocolate and liquid cocoa intake on endothelial function and blood pressure in overweight adults. **DESIGN:** Randomized, placebo-controlled, single-blind crossover trial of 45 healthy adults [mean age: 53 y; mean body mass index (in kg/m<sup>2</sup>): 30]. In phase 1, subjects were randomly assigned to consume a solid dark chocolate bar (containing 22 g cocoa powder) or a cocoa-free placebo bar (containing 0 g cocoa powder). In phase 2, subjects were randomly assigned to consume sugar-free cocoa (containing 22 g cocoa powder), sugared cocoa (containing 22 g cocoa powder), or a placebo (containing 0 g cocoa powder). **RESULTS:** Solid dark chocolate and liquid cocoa ingestion improved endothelial function (measured as flow-mediated dilatation) compared with placebo (dark chocolate: 4.3 +/- 3.4% compared with -1.8 +/- 3.3%;  $P < 0.001$ ; sugar-free and sugared cocoa: 5.7 +/- 2.6% and 2.0 +/- 1.8% compared with -1.5 +/- 2.8%;  $P < 0.001$ ). Blood pressure decreased after the ingestion of dark chocolate and sugar-free cocoa compared with placebo (dark chocolate: systolic, -3.2 +/- 5.8 mm Hg compared with 2.7 +/- 6.6 mm Hg;  $P < 0.001$ ; and diastolic, -1.4 +/- 3.9 mm Hg compared with 2.7 +/- 6.4 mm Hg;  $P = 0.01$ ; sugar-free cocoa: systolic, -2.1 +/- 7.0 mm Hg compared with 3.2 +/- 5.6 mm Hg;  $P < 0.001$ ; and diastolic: -1.2 +/- 8.7 mm Hg compared with 2.8 +/- 5.6 mm Hg;  $P = 0.014$ ). Endothelial function improved significantly more with sugar-free than with regular cocoa (5.7 +/- 2.6% compared with 2.0 +/- 1.8%;  $P < 0.001$ ). **CONCLUSIONS:** The acute ingestion of both solid dark chocolate and liquid cocoa improved endothelial function and lowered blood pressure in overweight adults. Sugar content may

attenuate these effects, and sugar-free preparations may augment them.

Am J Clin Nutr. 2008 Jul;88(1):58-63

### **GLISODIN, A VEGETAL SOD WITH GLIADIN, AS PREVENTATIVE AGENT VS. ATHEROSCLEROSIS, AS CONFIRMED WITH CAROTID ULTRASOUND-B IMAGING.**

Prevention of cardiovascular disease should target high-risk subjects based on genetic/familial factors, blood chemistry, blood pressure, body mass index (BMI), and a history of/or current cigarette smoking. We selected active adults (n=76) aged 30-60 and investigated these risk factors, in order to recommend preventive measures. Another interesting variable is the preclinical status or atheroma of the arterial (carotid) wall or lumen. We also investigated the presence of oxidative stress in, and the anti-oxidant status of these subjects. We studied the anti-oxidative efficacy of superoxide dismutase (SOD) and variations of malondialdehyde (MDA). Supplementation with GliSODin, a vegetal SOD associated with gliadin, was effective in controlling the thickness of the carotid artery intima and media layers as measured by ultrasonography-B. We could demonstrate the preventive efficacy of GliSODin at a preclinical stage in subjects with risk factors of cardiovascular disease.

Eur Ann Allergy Clin Immunol. 2007 Feb;39(2):45-50

### **COCOA REDUCES BLOOD PRESSURE AND INSULIN RESISTANCE AND IMPROVES ENDOTHELIUM-DEPENDENT VASODILATION IN HYPERTENSIVES.**

Consumption of flavanol-rich dark chocolate (DC) has been shown to decrease blood pressure (BP) and insulin resistance in healthy subjects, suggesting similar benefits in patients with essential hypertension (EH). Therefore, we tested the effect of DC on 24-hour ambulatory BP, flow-mediated dilation (FMD), and oral glucose tolerance tests (OGTTs) in patients with EH. After a 7-day chocolate-free run-in phase, 20 never-treated, grade I patients with EH (10 males; 43.7±7.8 years) were randomized to receive either 100 g per day DC (containing 88 mg flavanols) or 90 g per day flavanol-free white chocolate (WC) in an isocaloric manner for 15 days. After a second 7-day chocolate-free period, patients were crossed over to the other treatment. Noninvasive 24-hour ambulatory BP, FMD, OGTT, serum cholesterol, and markers of vascular inflammation were evaluated at the end of each treatment. The homeostasis model assessment of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), and insulin sensitivity index (ISI) were calculated from OGTT values. Ambulatory BP decreased after DC (24-hour systolic BP -11.9±7.7 mm Hg, P<0.0001; 24-hour diastolic BP -8.5±5.0 mm Hg, P<0.0001) but not WC. DC but not WC decreased HOMA-IR (P<0.0001), but it improved QUICKI, ISI, and FMD. DC also decreased serum LDL cholesterol (from 3.4±0.5 to 3.0±0.6 mmol/L; P<0.05). In summary, DC decreased BP and serum LDL cholesterol, improved FMD, and ameliorated insulin sensitivity in hypertensives. These results suggest that, while balancing total calorie intake, flavanols from cocoa products may provide some cardiovascular benefit if included as part of a healthy diet for patients with EH.

Hypertension. 2005 Aug;46(2):398-405

Breast cancer

**TARGETS OF VITAMIN D RECEPTOR SIGNALING IN THE MAMMARY GLAND.**

Since the discovery of the vitamin D receptor (VDR) in mammary cells, the role of the vitamin D signaling pathway in normal glandular function and in breast cancer has been extensively explored. In vitro studies have shown that the VDR ligand, 1,25-dihydroxyvitamin D (1,25D), modulates key proteins involved in signaling proliferation, differentiation, and survival of normal mammary epithelial cells. Similar anti-proliferative and pro-differentiating effects of 1,25D have been observed in VDR-positive breast cancer cells, indicating that transformation per se does not abolish vitamin D signaling. However, many transformed breast cancer lose sensitivity to 1,25D secondary to alterations in vitamin D metabolizing enzymes or downregulation of VDR function. Over the years, our laboratory has focused on three general areas: (1) defining mechanisms of vitamin D-mediated apoptosis in breast cancer cells, (2) examining changes in the vitamin D signaling pathway during transformation, including the development of vitamin D resistance, and (3) using mouse models to study the impact of the VDR on growth regulatory pathways in the context of development and tumorigenesis in vivo. Recent developments include detection of megalin-mediated uptake of vitamin D-binding protein (DBP) and identification of CYP27B1 and CYP24 metabolizing enzymes in mammary cells, demonstration of precocious mammary gland development in VDR-null mice, and identification of novel pathways triggered by 1,25D during apoptosis. Our preclinical studies have been complemented by emerging data from other groups suggesting that human breast cancer may be influenced by VDR genotype and vitamin D status. Collectively, these studies have reinforced the need to further define the regulation and function of the vitamin D pathway in cells in relation to prevention and treatment of breast cancer.

J Bone Miner Res. 2007 Dec;22 Suppl 2:V86-90

**VITAMIN D INTAKE AND BREAST CANCER RISK IN POSTMENOPAUSAL WOMEN: THE IOWA WOMEN'S HEALTH STUDY.**

Vitamin D, a prosteroid hormone with anti-proliferative and pro-differentiation activity, is thought to act as a cancer chemopreventive agent. This study evaluated the association between vitamin D intake and breast cancer risk among women in a large prospective cohort study. A total of 34,321 postmenopausal women who had completed a questionnaire that included diet and supplement use were followed for breast cancer incidence from 1986 to 2004. Adjusted relative risks (RR) for breast cancer were calculated for dietary, supplemental, and total vitamin D intake among all women. The adjusted RR of breast cancer for women consuming >800 IU/day versus <400 IU/day total vitamin D was 0.89 (95% CI: 0.77-1.03). RRs were stronger among women with negative than positive ER or PR status. The association of high vitamin D intake with breast cancer was strongest in the first 5 years after baseline dietary assessment (RR = 0.66; 95% CI: 0.46-0.94 compared with lowest-intake group), and diminished over time. Changes in vitamin D intake over time might have contributed to the diminished association observed in later years. Vitamin D intake of >800 IU/day appears to be associated with a small decrease in risk of breast cancer among postmenopausal women. Studies evaluating all sources of vitamin D, especially sun exposure, are needed to fully understand the association between vitamin D and breast cancer risk.

Cancer Causes Control. 2007 Sep;18(7):775-82

**VITAMIN D AND PREVENTION OF BREAST CANCER: POOLED ANALYSIS.**

**BACKGROUND:** Inadequate photosynthesis or oral intake of Vitamin D are associated with high incidence and mortality rates of breast cancer in ecological and observational studies, but the dose-response relationship in individuals has not been adequately studied. **METHODS:** A literature search for all studies that reported risk by of breast cancer by quantiles of 25(OH)D identified two studies with 1,760 individuals. Data were pooled to assess the dose-response association between serum 25(OH)D and risk of breast cancer. **RESULTS:** The medians of the pooled quintiles of serum 25(OH)D were 6, 18, 29, 37, and 48 ng/ml. Pooled odds ratios for breast cancer from lowest to highest quintile, were 1.00, 0.90, 0.70, 0.70, and 0.50 (p trend<0.001). According to the pooled analysis, individuals with serum 25(OH)D of approximately 52 ng/ml had 50% lower risk of breast cancer than those with serum <13 ng/ml. This serum level corresponds to intake of 4,000 IU/day. This exceeds the National Academy of Sciences upper limit of 2,000 IU/day. A 25(OH)D level of 52 ng/ml could be maintained by intake of 2,000 IU/day and, when appropriate, about 12 min/day in the sun, equivalent to oral intake of 3,000 IU of Vitamin D(3). **CONCLUSIONS:** Intake of 2,000 IU/day of Vitamin D(3), and, when possible, very moderate exposure to sunlight, could raise serum 25(OH)D to 52 ng/ml, a level associated with reduction by 50% in incidence of breast cancer, according to observational studies.

### **(-)-EPIGALLOCATECHIN-3-GALLATE DOWNREGULATES ESTROGEN RECEPTOR ALPHA FUNCTION IN MCF-7 BREAST CARCINOMA CELLS.**

**BACKGROUND:** (-)-Epigallocatechin-3-gallate (EGCG) is the most active catechin present in green tea, demonstrated to have chemopreventive action and to kill cancer cells selectively. As a previous study found that catechins could compete with 17-beta-estradiol for binding to estrogen receptor alpha (ERalpha), we asked whether EGCG could regulate ERalpha action. **METHODS:** We used MCF-7, a breast carcinoma cell line having a high level of ERalpha expression. The cells were treated with various EGCG concentrations and cell viability was evaluated by MTT assay. ERalpha and pS2 expression were analyzed by RT-PCR after RNA extraction. To better define EGCG action in relation to ERalpha, we studied EGCG cytotoxicity on MCF-7 resistant to tamoxifen (MCF-7tam), MCF-7 treated with 10(-7)M ICI 182,780 for 8 days and on MDA-MB-231, a cell line that lacked ERalpha by flow cytometry (FCM). **RESULTS:** Both ERalpha and pS2 mRNA were expressed in samples treated with low EGCG concentration (30 microg/ml). At this concentration, no cell change was detectable. In contrast, pS2 expression was lost in samples treated with 100 microg/ml EGCG for 24h, indicating ERalpha alteration. EGCG cytotoxicity was lower when ERalpha was not present (MDA-MB-231) or inactivated (by tamoxifen or ICI 182,780). **CONCLUSIONS:** Functionally active ERalpha may have a role in EGCG cytotoxicity, increasing the sensitivity to the drug. As higher EGCG concentrations also killed cells resistant to tamoxifen or treated by 10(-7)M ICI 182,780, EGCG ought to be better investigated in breast carcinoma cells treated with drugs targeted to steroid receptors, as a potential complement of therapy.

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### **RISK ASSESSMENT FOR VITAMIN D.**

The objective of this review was to apply the risk assessment methodology used by the Food and Nutrition Board (FNB) to derive a revised safe Tolerable Upper Intake Level (UL) for vitamin D. New data continue to emerge regarding the health benefits of vitamin D beyond its role in bone. The intakes associated with those benefits suggest a need for levels of supplementation, food fortification, or both that are higher than current levels. A prevailing concern exists, however, regarding the potential for toxicity related to excessive vitamin D intakes. The UL established by the FNB for vitamin D (50 microg, or 2,000 IU) is not based on current evidence and is viewed by many as being too restrictive, thus curtailing research, commercial development, and optimization of nutritional policy. Human clinical trial data published subsequent to the establishment of the FNB vitamin D UL published in 1997 support a significantly higher UL. We present a risk assessment based on relevant, well-designed human clinical trials of vitamin D. Collectively, the absence of toxicity in trials conducted in healthy adults that used vitamin D dose > or = 250 microg/d (10,000 IU vitamin D3) supports the confident selection of this value as the UL.

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### **CURCUMIN AND CANCER: AN "OLD-AGE" DISEASE WITH AN "AGE-OLD" SOLUTION.**

Cancer is primarily a disease of old age, and that life style plays a major role in the development of most cancers is now well recognized. While plant-based formulations have been used to treat cancer for centuries, current treatments usually involve poisonous mustard gas, chemotherapy, radiation, and targeted therapies. While traditional plant-derived medicines are safe, what are the active principles in them and how do they mediate their effects against cancer is perhaps best illustrated by curcumin, a derivative of turmeric used for centuries to treat a wide variety of inflammatory conditions. Curcumin is a diferuloylmethane derived from the Indian spice, turmeric (popularly called "curry powder") that has been shown to interfere with multiple cell signaling pathways, including cell cycle (cyclin D1 and cyclin E), apoptosis (activation of caspases and down-regulation of antiapoptotic gene products), proliferation (HER-2, EGFR, and AP-1), survival (PI3K/AKT pathway), invasion (MMP-9 and adhesion molecules), angiogenesis (VEGF), metastasis (CXCR-4) and inflammation (NF-kappaB, TNF, IL-6, IL-1, COX-2, and 5-LOX). The activity of curcumin reported against leukemia and lymphoma, gastrointestinal cancers, genitourinary cancers, breast cancer, ovarian cancer, head and neck squamous cell carcinoma, lung cancer, melanoma, neurological cancers, and sarcoma reflects its ability to affect multiple targets. Thus an "old-age" disease such as cancer requires an "age-old" treatment.

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