

ABSTRACTS

Skin Care

Low molecular weight antioxidants and their role in skin ageing.

There is increasing evidence that reactive oxygen species play a pivotal role in the process of ageing. The skin, as the outermost barrier of the body, is exposed to various exogenous sources of oxidative stress, in particular UV-irradiation. These are believed to be responsible for the extrinsic type of skin ageing, termed photo-ageing. It therefore seems reasonable to try to increase levels of protective low molecular weight antioxidants through a diet rich in fruits and vegetables or by direct topical application. Indeed, various in vitro and animal studies have proved that low molecular weight antioxidants, especially vitamins C and E, ascorbate and tocopherol, as well as lipoic acid, exert protective effects against oxidative stress. However, controlled long-term studies on the efficacy of low molecular weight antioxidants in the prevention or treatment of skin ageing in humans are still lacking.

Clin Exp Dermatol. 2001 Oct;26(7):578-82

Photoaging is associated with protein oxidation in human skin in vivo.

There is increasing evidence for the generation of reactive oxygen species in skin upon ultraviolet exposure, but little is known about their pathophysiologic relevance in human skin in vivo. We hypothesized that chronic and acute photodamage is mediated by depleted antioxidant enzyme expression and increased oxidative protein modifications. Biopsies from patients with histologically confirmed solar elastosis, from non-ultraviolet-exposed sites of age-matched controls, and from young subjects were analyzed. To evaluate the influence of acute ultraviolet exposures, buttock skin of 12 healthy subjects was irradiated repetitively on 10 d with a solar simulator and compared intraindividually to non-ultraviolet-treated contralateral sites. The antioxidant enzymes catalase, copper-zinc superoxide dismutase, and manganese superoxide dismutase were investigated by immunohistochemistry. Protein carbonyls were analyzed by immunohistochemical and immunoblotting techniques in human skin and in cell models. Whereas overall expression of antioxidant enzymes was very high in the epidermis, low baseline levels were found in the dermis. In photoaged skin, a significant depletion of antioxidant enzyme expression was observed within the stratum corneum and in the epidermis. Importantly, an accumulation of oxidatively modified proteins was found specifically within the upper dermis of photoaged skin. Upon acute ultraviolet exposure of healthy subjects, depleted catalase expression and increased protein oxidation were detected. Exposures of keratinocytes and fibroblasts to ultraviolet B, ultraviolet A, and H₂O₂ led to dose-dependent protein oxidation and thus confirmed in vivo results. In conclusion, the correlation between photodamage and protein oxidation was demonstrated for the first time, which hence may be a relevant pathophysiologic factor in photoaging.

J Invest Dermatol. 2002 Apr;118(4):618-25

Skin photodamage and lifetime photoprotection.

Ultraviolet (UV) radiation is a very small part of the electromagnetic radiation spectrum, released and transported from the source in the form of photons. Disposal of these photons within the skin causes cutaneous photodamage, which leads to clinical, histologic, and biochemical changes. Aging is a complex process characterized by cellular attrition, decreased cellular reserve capacity, and compromised ability to perform normal cellular function. Intrinsic aging, which steadily develops with time, is linked to chronologic age; it is the result of a genetic program. Photoaging, on the other hand, develops as a consequence of UV radiation-induced degenerative changes in the skin. Intrinsic aging is a universal, inevitable process, whereas photoaging is neither universal nor inevitable and can be prevented. UV radiation can also suppress the immune system in both local and systemic way and lead to simultaneous and sequential biochemical events that ultimately cause photocarcinogenesis. Therefore, everyday use of products that protect against UV radiation is necessary to prevent acute and long-term photodamage (clinical and cellular changes) leading to photoaging, photoimmunosuppression, and photocarcinogenesis.

Acta Dermatovenerol Croat. 2003;11(1):32-40

Topical applications of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice.

SKH-1 hairless mice were irradiated with ultraviolet B (UVB) twice weekly for 20 weeks. These tumor-free mice, which had a high risk of developing skin tumors during the next several months, were then treated topically with caffeine (6.2 micromol) or (-)-epigallocatechin gallate (EGCG; 6.5 micromol) once a day 5 days a week for 18 weeks in the absence of further treatment with UVB. Topical applications of caffeine to these mice decreased the number of nonmalignant and malignant skin tumors per mouse by 44% and 72%, respectively. Topical applications of EGCG decreased the number of nonmalignant and malignant tumors per

mouse by 55% and 66%, respectively. Immunohistochemical analysis showed that topical applications of caffeine or EGCG increased apoptosis as measured by the number of caspase 3-positive cells in nonmalignant skin tumors by 87% or 72%, respectively, and in squamous cell carcinomas by 92% or 56%, respectively, but there was no effect on apoptosis in nontumor areas of the epidermis. Topical applications of caffeine or EGCG had a small inhibitory effect on proliferation in nonmalignant tumors as measured by BrdUrd labeling (16-22%), and there was also a similar, but nonsignificant, inhibitory effect on proliferation in malignant tumors. The results suggest a need for further studies to determine whether topical applications of caffeine or EGCG can inhibit sunlight-induced skin cancer in humans.

Proc Natl Acad Sci U S A. 2002 Sep 17;99(19):12455-60. Epub 2002 Aug 30

Green tea polyphenols: DNA photodamage and photoimmunology.

Green tea is a popular beverage consumed worldwide. The epicatechin derivatives, which are commonly called 'polyphenols', are the active ingredients in green tea and possess antioxidant, anti-inflammatory and anti-carcinogenic properties. Studies conducted by our group on human skin have demonstrated that green tea polyphenols (GTP) prevent ultraviolet (UV)-B-induced cyclobutane pyrimidine dimers (CPD), which are considered to be mediators of UVB-induced immune suppression and skin cancer induction. GTP treated human skin prevented penetration of UV radiation, which was demonstrated by the absence of immunostaining for CPD in the reticular dermis. The topical application of GTP or its most potent chemopreventive constituent (-)-epigallocatechin-3-gallate (EGCG) prior to exposure to UVB protects against UVB-induced local as well as systemic immune suppression in laboratory animals. Additionally, studies have shown that EGCG treatment of mouse skin inhibits UVB-induced infiltration of CD11b+ cells. CD11b is a cell surface marker for activated macrophages and neutrophils, which are associated with induction of UVB-induced suppression of contact hypersensitivity responses. EGCG treatment also results in reduction of the UVB-induced immunoregulatory cytokine interleukin (IL)-10 in skin as well as in draining lymph nodes, and an elevated amount of IL-12 in draining lymph nodes. These in vivo observations suggest that GTPs are photoprotective, and can be used as pharmacological agents for the prevention of solar UVB light-induced skin disorders associated with immune suppression and DNA damage.

J Photochem Photobiol B. 2001 Dec 31;65(2-3):109-14

Preformulation study of epigallocatechin gallate, a promising antioxidant for topical skin cancer prevention.

Epigallocatechin gallate (EGCG) is a potent polyphenolic antioxidant extracted from green tea. Due to its antimutagenic and antitumor activities, it is a promising candidate for use in topical formulations for skin cancer prevention. The overall goal of this study was therefore to determine the influence of several factors on the stability of EGCG in solution to obtain information that would facilitate the subsequent development of topical formulations. Our first objective was to determine the influence of pH, temperature, and ionic strength on the aqueous stability of EGCG. A second objective was to determine the stability of EGCG in various solvents in the presence and absence of different antioxidants. A simple and rapid stability indicating high-performance liquid chromatography assay for EGCG was developed. Stability studies were performed in 0.05 M aqueous buffers at pH 3, 5, 7, and 9 at 4, 25, and 50 degrees C. The effect of ionic strength on EGCG stability was evaluated in 0.05 M acetate buffer, pH 5, adjusted to the desired ionic strength with sodium chloride. An accelerated stability study of EGCG was performed at 50 degrees C in the organic solvents glycerin and Transcutol P in the presence of antioxidants. The degradation of EGCG increased rapidly as temperature and solution pH were increased. Ionic strength increases also caused an accelerated degradation. The solution stability of EGCG was prolonged in glycerin and Transcutol P compared with an aqueous environment. The addition of 0.1% concentrations of several antioxidants in combination with 0.025% EDTA caused variable effects on EGCG stability. Butylated hydroxytoluene in glycerin produced the greatest stability improvement for EGCG. The t(90) (time for 10% degradation to occur) was 76.1 days at 50 degrees C. It can be concluded that glycerin-based vehicles are suitable for stabilizing EGCG.

J Pharm Sci. 2002 Jan;91(1):111-6

ABSTRACTS

Natural Estrogen

Effects of genistein on cell proliferation and cell cycle arrest in nonneoplastic human mammary epithelial cells: involvement of Cdc2, p21(waf/cip1), p27(kip1), and Cdc25C expression.

Genistein, a soy isoflavone, has been reported to inhibit the multiplication of numerous neoplastic cells, including those in the breast. However, there is limited information on the effect of genistein on nonneoplastic human breast cells. In the present studies, genistein inhibited proliferation of, and DNA synthesis in, the nonneoplastic human mammary epithelial cell line MCF-10F with an IC(50) of approximately 19-22 microM, and caused a reversible G2/M block in cell cycle progression. Genistein treatment (45 microM) increased the phosphorylation of Cdc2 by 3-fold, decreased the activity of Cdc2 by 70% after 8 hr, and by 24 hr reduced the expression of Cdc2 by 70%. In addition, genistein enhanced the expression of the cell cycle inhibitor p21 (waf/cip1) by 10- to 15-fold, increased p21(waf/cip1) association with Cdc2 by 2-fold, and increased the expression of the tumor suppressor p53 by 2.8-fold. Genistein did not alter the expression of p27(kip1) significantly. Furthermore, genistein inhibited the expression of the cell cycle-associated phosphatase Cdc25C by 80%. From these results, we conclude that genistein inhibits the growth of nonneoplastic MCF-10F human breast cells by preventing the G2/M phase transition, induces the expression of the cell cycle inhibitor p21(waf/cip1) as well as its interaction with Cdc2, and inhibits the activity of Cdc2 in a phosphorylation-related manner. Down-regulation of the cell cycle-associated phosphatase Cdc25C combined with up-regulation of p21(waf/cip1) expression appear to be important mechanisms by which genistein decreases Cdc2 kinase activity and causes G2 cell cycle arrest.

Biochem Pharmacol. 2001 Apr 15;61(8):979-89

Dietary phytoestrogens and vascular function in postmenopausal women: a cross-sectional study.

OBJECTIVE: To investigate the effects of low levels of intake of phytoestrogens in Western habitual diet on vascular function. **DESIGN:** A cross-sectional study. **SETTING:** A population-based study. **PARTICIPANTS:** A total of 301 postmenopausal women aged 60-75 years living in The Netherlands. **DETERMINANT:** Dietary phytoestrogen intake as assessed using a food frequency questionnaire covering the year prior to enrollment. **MAIN OUTCOME MEASURES:** Blood pressure, hypertension, endothelial function and ankle brachial index. **RESULTS:** The median isoflavone intake was 0.2 mg in the lowest tertile and 11.4 mg in the highest tertile. Median lignan intake was 0.8 and 2.2 mg, respectively. No associations were found for higher intake of isoflavones, systolic and diastolic blood pressures, ankle-arm blood pressure index, endothelial function or hypertension. For lignans no association was found for ankle-arm blood pressure index or endothelial function, but we did observe lower systolic and diastolic blood pressures and a lower prevalence of hypertension (systolic blood pressure difference T3-T1, -11.2 mmHg, 95% confidence interval = -17.8 to -4.5, P for trend = 0.001; diastolic blood pressure difference T3-T1, -3.6 mmHg, 95% confidence interval = -7.8 to 0.6, P for trend = 0.08; and prevalence of hypertension, odds ratio T3 versus T1 = 0.41, 95% confidence interval = 0.22-0.76, P for trend over tertiles = 0.004). **CONCLUSION:** The results of this study suggest a protective effect of dietary lignan intake on blood pressure and hypertension, even at low levels.

J Hypertens. 2004 Jul;22(7):1381-8

Phyto-oestrogen excretion and rate of bone loss in postmenopausal women.

OBJECTIVE: The hypothesis was tested that the rate of postmenopausal bone loss is inversely associated with long-term urinary excretion of phyto-oestrogens, as a marker of habitual dietary intake. **DESIGN:** Secondary analysis of a 10-year follow-up study (1979-1989) among postmenopausal women in the Netherlands. **SUBJECTS:** From the original population of 154 women, 32 women were selected with an annual rate of radial bone loss of < or = 0.5% over the first 5 years of the study and 35 women with a rate of > or = 2.5% per year. **METHODS:** The isoflavonoids genistein, daidzein and equol, and the lignan enterolactone were determined by gas chromatography mass spectrometry in aggregate samples from annually collected urine samples. Cortical bone density of the radius had previously been measured annually by single-photon absorptiometry. **RESULTS:** Excretion of isoflavonoids did not differ between both groups, although in multivariate analysis equol excretion was weakly positively associated with rate of bone loss in the 5 years after the menopause. Enterolactone excretion was significantly higher in the group with high rate of bone loss. This positive association remained in multivariate linear regression analysis after adjustment for age, years since menopause, body mass index and intake of calcium, vegetable protein and dietary fibre. **CONCLUSIONS:** Enterolactone excretion is likely to be an indicator of consumption of grains and legumes; it is not clear whether the observed positive association with rate of bone loss is a causal one. Our results do not support a preventive effect of low, unsupplemented dietary intake of phyto-oestrogens on postmenopausal cortical bone loss. However, no conclusions can be drawn about effects of higher doses of phyto-oestrogens.

Eur J Clin Nutr. 1998 Nov;52(11):850-5

Soy intake and the maintenance of peak bone mass in Hong Kong Chinese women.

Our previous study on bone health among premenopausal women showed that bone mass consolidation is attained by the early 30s, and small loss of spinal bone mineral density (SBMD) occurs soon after peak bone mass attainment. Recent interest has been shown in the potential beneficial effects of phytoestrogens on bone health. However, data are lacking, particularly in Asian women. This study aims to investigate the effect of soy isoflavones intake on the maintenance of peak bone mass in a cohort of 132 women aged 30-40 years who were followed up for 3 years. Baseline measurements of SBMD (L2-L4) were obtained using dual-energy X-ray densitometry, and dietary intake of soy foods and other key nutrients, including dietary calcium, were obtained through a quantitative food frequency method. Information on body measurements; physical activity (PA), weight-bearing activity in particular; age of menarche; and number of pregnancies were obtained at baseline. Repeated measurements of SBMD were obtained yearly for a further 3 years with an average follow-up time of 38 months. Analyses were performed on 116 subjects with at least three SBMD measurements (at baseline, 3-year follow-up, and at least one measurement during follow-up). The individual SBMD regression slope was computed for each of the subjects. Soy isoflavones consumption was categorized as quartiles of intake. We observed a significant difference in the SBMD individual regression slopes between women belonging to the fourth and first soy isoflavones intake quartiles. The positive effect of soy isoflavones on SBMD remained after adjusting for age and body size (height, weight, and bone area). Multiple linear regression analysis including the other known covariates (lean body mass, PA, energy adjusted calcium, and follow-up time) showed that soy isoflavones, together with these variables, accounted for 24% of the variances of the SBMD individual regression slope. This longitudinal study shows that soy intake had a significant effect on the maintenance of SBMD in women aged 30-40 years. The effects of phytoestrogens on bone health should be explored further in a population with habitual dietary soy but low calcium intake.

J Bone Miner Res . 2001 Jul;16(7):1363-9

High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not premenopausal women.

Animal studies demonstrated that phytoestrogen had a protective effect against bone loss after ovariectomy. However, data on dietary phytoestrogen intake as well as its relationship with bone mineral density (BMD) in human are not available. Six hundred fifty southern Chinese women, aged 19 to 86 yr, were recruited to determine their dietary phytoestrogen intake by a food frequency questionnaire. BMDs at the lumbar spine and hip region were measured using dual energy x-ray absorptiometry. The subjects were analyzed according to various tertiles of phytoestrogen intake. Among the postmenopausal women (n = 357), significant differences in the lumbar spine (L2-4) BMD (0.820 +/- 0.145 vs. 0.771 +/- 0.131 g/cm², P < 0.05) and Ward's triangle BMD (0.450 +/- 0.151 vs. 0.415 +/- 0.142 g/cm²; P < 0.05) were found between the highest and lowest intake of isoflavone after adjusting for age, height, weight, years since menopause, smoking, alcohol consumption, HRT usage, and daily calcium intake. Women with the highest intake of isoflavone had significantly lower levels of serum PTH (19.38 +/- 14.61 vs. 26.56 +/- 11.19 pg/ml; P < 0.05), osteocalcin (4.95 +/- 3.61 vs. 6.69 +/- 5.05 mg/liter; P = 0.05), and urinary N-telopeptide (34.18 +/- 25.31 vs. 49.66 +/- 41.00 nmol bone collagen equivalents/mmol creatinine; P < 0.05) when compared with those with the lowest intake of isoflavone. No association between dietary phytoestrogen intake and BMDs was seen in the premenopausal women with high endogenous E (n = 293). In conclusion, postmenopausal women with habitually high intake of dietary isoflavone are associated with higher BMD values at both the spine and hip region. Customarily high isoflavone intake may help to reverse the state of secondary hyperparathyroidism associated with E withdrawal and hence lower the rate of bone turnover in postmenopausal women.

J Clin Endocrinol Metab. 2001 Nov;86(11):5217-21

Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study.

The natural isoflavone phytoestrogen genistein has been shown to stimulate osteoblastic bone formation, inhibit osteoclastic bone resorption, and prevent bone loss in ovariectomized rats. However, no controlled clinical trial has been performed so far to evaluate the effects of the phytoestrogen on bone loss in postmenopausal women. We performed a randomized double-blind placebo-controlled study to evaluate and compare with hormone-replacement therapy (HRT) the effect of the phytoestrogen genistein on bone metabolism and bone mineral density (BMD) in postmenopausal women. Participants were 90 healthy ambulatory women who were 47-57 years of age, with a BMD at the femoral neck of <0.795 g/cm². After a 4-week stabilization on a standard fat-reduced diet, participants of the study were randomly assigned to receive continuous HRT for 1 year (n = 30; 1 mg of 17beta-estradiol [E2] combined with 0.5 mg of norethisterone acetate), the phytoestrogen genistein (n = 30; 54 mg/day), or placebo (n = 30). Urinary excretion of pyridinoline (PYR) and deoxypyridinoline (DPYR) was not significantly modified by placebo administration either at 6 months or at 12 months. Genistein treatment significantly reduced the excretion of pyridinium cross-links at 6 months (PYR = -54 +/- 10%; DPYR = -55 +/- 13%; p < 0.001) and 12 months (PYR = -42 +/- 12%; DPYR = -44 +/- 16%; p < 0.001). A similar and not statistically different decrease in excretion of pyridinium cross-links was also observed in the postmenopausal women randomized to receive HRT. Placebo administration did not change the serum levels of the bone-specific ALP (B-ALP) and osteocalcin (bone Gla protein [BGP]). In contrast, administration of genistein markedly increased serum B-ALP and BGP either at 6 months (B-ALP = 23 +/- 4%; BGP = 29 +/- 11%; p < 0.005) or at 12 months (B-ALP = 25 +/- 7%; BGP = 37 +/- 16%; p < 0.05). Postmenopausal women treated with HRT had, in contrast, decreased serum B-ALP and BGP levels either at 6 months (B-ALP = -17 +/- 6%; BGP = -20 +/- 9%; p < 0.001) or 12 months (B-ALP = -20 +/- 5%; BGP = -22 +/- 10%; p < 0.001). Furthermore, at the end of the experimental period, genistein and HRT significantly increased BMD in the femur (femoral neck: genistein = 3.6 +/- 3%, HRT = 2.4 +/- 2%, placebo = -0.65 +/- 0.1%, and p < 0.001) and lumbar spine (genistein = 3 +/-

2%, HRT = 3.8 +/- 2.7%, placebo = -1.6 +/- 0.3%, and p < 0.001). This study confirms the genistein-positive effects on bone loss already observed in the experimental models of osteoporosis and indicates that the phytoestrogen reduces bone resorption and increases bone formation in postmenopausal women.

J Bone Miner Res. 2002 Oct;17(10):1904-12

Relationships of urinary phyto-oestrogen excretion to BMD in postmenopausal women.

OBJECTIVE: Phyto-oestrogens are plant compounds with both oestrogenic and anti-oestrogenic properties. However, it is not known whether natural phyto-oestrogens are beneficial or harmful in human osteoporosis. This study was performed to investigate the relationships between urinary phyto-oestrogens and bone mineral density (BMD) in Korean postmenopausal women. **DESIGN:** The subjects were classified into osteoporotic, osteopenic and normal groups according to their BMD as defined by WHO criteria. We compared the urinary phyto-oestrogens of each group and studied whether urinary phyto-oestrogens correlate with BMD. **PATIENTS:** The subjects were 75 Korean postmenopausal women with ages ranging from 52 to 65 years (mean 58 +/- 1.1 years). Mean number of years after menopause was 7.3 +/- 1.3. **MEASUREMENTS:** Twenty-four-hour urinary phyto-oestrogens were measured by gas chromatography-mass spectrometry (GCMS) and BMD by dual-energy X-ray absorptiometry (DXA, Lunar Expert-XL, Lunar Co., WI, USA). **RESULTS:** In Korean postmenopausal women, urinary enterolactone (1.46 +/- 1.11 micromol/day) was lower and daidzein (2.59 +/- 3.25 micromol/day) was higher than in western women, and both levels were comparable to those in Japanese women. Daily urinary excretion of genistein and apigenin were 1.09 +/- 0.912 and 0.48 +/- 0.40 micromol/day, respectively. In subjects with osteoporosis, urinary enterolactone was lower (P < 0.05) but apigenin was significantly higher (P < 0.05) than in the controls. BMD of L2-L4 correlated positively with urinary enterolactone (r = 0.388, P < 0.01), and BMD of the femoral neck and Ward's triangle correlated positively with urinary enterolactone (r = 0.271, P < 0.05 and r = 0.322, P < 0.05) but negatively with apigenin (r = -0.412, P < 0.01 and r = -0.395, P < 0.01). By multiple stepwise regression, the variables associated with spinal BMD were age, the amount of urinary apigenin and body mass index (BMI). The variables associated with femoral neck BMD were age and urinary apigenin. **CONCLUSIONS:** From these results, we conclude that urinary phyto-oestrogens, especially enterolactone and apigenin, are related to BMD in Korean postmenopausal women. Our results also suggest the possibility that phyto-oestrogens have differential effects on bone density. Further studies are needed to clarify the exact biological roles of phyto-oestrogenic components on bone metabolism.

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