

## ABSTRACTS

### Arthritis

#### **Licofelone (ML-3000), a dual inhibitor of 5-lipoxygenase and cyclooxygenase, reduces the level of cartilage chondrocyte death in vivo in experimental dog osteoarthritis: inhibition of pro-apoptotic factors.**

**OBJECTIVE:** To evaluate in vivo therapeutic efficacy of licofelone, a novel competitive dual inhibitor of 5-lipoxygenase (5-LOX) and cyclooxygenase (COX) in chondrocyte death in the canine ligament transection model of osteoarthritis (OA), and to explore its effect on factors involved in the apoptotic phenomenon, i.e., caspase-3, COX-2 and inducible nitric oxide synthase (iNOS).

**METHODS:** Cartilage specimens were obtained from three experimental groups of dogs: Group 1, dogs subjected to sectioning of the anterior cruciate ligament of the right knee and given placebo treatment; Groups 2 and 3, operated dogs that received oral treatment with licofelone (2.5 or 5.0 mg/kg/day, respectively) for eight weeks starting immediately after surgery. All dogs were killed eight weeks postsurgery. The cartilage level of chondrocyte death was detected by TUNEL reaction. Cartilage distribution of caspase-3, COX-2 and iNOS was documented by immunohistochemistry using specific antibodies, and other levels were quantified by morphometric analysis. **RESULTS:** In cartilage specimens from placebo treated dogs a large number of chondrocytes in the superficial layers stained positive for TUNEL reaction. Treatment with therapeutic concentrations of licofelone (2.5 and 5.0 mg/kg/day) markedly reduced the level of chondrocyte apoptosis to the same extent in both therapeutic groups ( $p < 0.0001$ ,  $p < 0.002$ , respectively). In these groups, the levels of caspase-3, COX-2 and iNOS in cartilage from both condyles and plateaus were also significantly decreased ( $p < 0.0001$ ,  $p < 0.0001$ ,  $p < 0.0002$ , respectively) compared to the control (placebo) group. **CONCLUSION:** Licofelone is an effective treatment in vivo, capable of reducing the level of OA chondrocyte death. This effect is likely mediated by a decrease in the level of caspase-3 activity, which may be related to the reduced production of two major factors involved in chondrocyte apoptosis, NO and prostaglandin E2. These findings may explain some of the mechanisms by which licofelone reduces the progression of experimental OA.

*J Rheumatol. 2002 Jul;29(7):1446-53.*

#### **Differential regulation of prostaglandin E2 and thromboxane A2 production in human monocytes: implications for the use of cyclooxygenase inhibitors.**

There is an autocrine relationship between eicosanoid and cytokine synthesis, with the ratio of prostaglandin E2 (PGE2)/thromboxane A2 (TXA2) being one of the determinants of the level of cytokine synthesis. In monocytes, cyclooxygenase type 1 (COX-1) activity appears to favor TXA2 production and COX-2 activity appears to favor PGE2 production. This has led to speculation regarding possible linkage of COX isozymes with PGE and TXA synthase. We have studied the kinetics of PGE2 and TXA2 synthesis under conditions that rely on COX-1 or -2 activity. With small amounts of endogenously generated prostaglandin H2 (PGH2), TXA2 synthesis was greater than PGE2. With greater amounts of endogenously generated PGH2, PGE2 synthesis was greater than TXA2. Also, TXA synthase was saturated at lower substrate concentrations than PGE synthase. This pattern was observed irrespective of whether PGH2 was produced by COX-1 or COX-2 or whether it was added directly. Furthermore, the inhibition of eicosanoid production by the action of nonsteroidal anti-inflammatory drugs or by the prevention of COX-2 induction with the p38 mitogen-activated protein kinase inhibitor SKF86002 was greater for PGE2 than for TXA2. It is proposed that different kinetics of PGE synthase and TXA synthase account for the patterns of production of these eicosanoids in monocytes under a variety of experimental conditions. These properties provide an alternative explanation to notional linkage or compartmentalization of COX-1 or -2 with the respective terminal synthases and that therapeutically induced changes in eicosanoid ratios toward predominance of TXA2 may have unwanted effects in long-term anti-inflammatory and anti-arthritis therapy.

*J Immunol. 2000 Aug 1;165(3):1605-11*

#### **Thromboxane A2 induces leukotriene B4 synthesis that in turn mediates neutrophil diapedesis via CD 18 activation.**

Previous studies have indicated that thromboxane (Tx) and leukotriene (LT) B4 act synergistically to induce neutrophil (PMN) adhesion in the microvasculature. This study explores the ability of Tx to induce LTB4 synthesis, which then leads to activation of PMN and endothelial adhesion receptors. Tx-mimic (U46619, 1 microgram/ml) was administered into abraded skin chambers placed on the backs of rabbits ( $n = 6$ ). After three hours LTB4 was synthesized in the blister fluid at 385 pg/ml, a value higher than levels in saline-treated blisters, 10 pg/ml ( $P$  less than 0.05). The LTB4 generation following Tx-mimic was correlated ( $P$  less than 0.05,  $r = 0.70$ ) with neutrophil diapedesis. These averaged 645 PMN/mm<sup>3</sup>, values higher than saline values of 20 PMN/mm<sup>3</sup> ( $P$  less than 0.05). Intravenous (iv) treatment of other rabbits ( $n = 4$ ) with the lipoxygenase inhibitor diethylcarbamazine at 60 mg/kg, followed by 40 mg/kg/hr, prevented Tx-mimic-induced LTB4 synthesis (10 pg/ml) and diapedesis (19 PMN/mm<sup>3</sup>) (both  $P$

less than 0.05). In contrast, local administration of 3 ng of the protein synthesis inhibitor actinomycin D, to prevent expression of endothelial adhesion proteins, limited TNF- but not Tx-induced diapedesis. The data indicate that Tx-induced diapedesis is mediated by the generation of LTB4 and the activation of neutrophil CD 18 but not endothelial adhesion proteins.

*Microvasc Res.* 1991 May;41(3):367-75

### **Anti-cytokine therapy in chronic destructive arthritis.**

Tumor necrosis factor (TNF) and interleukin-1 (IL-1) are considered to be master cytokines in chronic, destructive arthritis. Therapeutic approaches in rheumatoid arthritis (RA) patients have so far focused mainly on TNF, which is a major inflammatory mediator in RA and a potent inducer of IL-1; anti-TNF therapy shows great efficacy in RA patients. However, it is not effective in all patients, nor does it fully control the arthritic process in affected joints of good responders. Directed therapy for IL-1, with IL-1 receptor antagonist, mainly reduces erosions and is marginally anti-inflammatory. It is as yet unclear whether the limited effect is akin to the RA process or linked to suboptimal blocking of IL-1. Analysis of cytokine patterns in early synovial biopsies of RA patients reveals a marked heterogeneity, with variable staining of TNF and IL-1 beta, indicative of TNF-independent IL-1 production in at least some patients. Evidence for this pathway emerged from experimental arthritides in rodents, and is summarized in this review. If elements of the models apply to the arthritic process in RA patients, it is necessary to block IL-1 beta in addition to TNF.

*Arthritis Res.* 2001;3(1):18-26. Epub 2000 Nov 10

### **Ex-vivo in-vitro inhibition of lipopolysaccharide stimulated tumor necrosis factor-alpha and interleukin-1 beta secretion in human whole blood by extractum urticae dioicae foliorum.**

An extract of *Urtica dioica* folium (IDS 23, Rheuma-Hek), monographed positively for adjuvant therapy of rheumatic diseases and with known effects in partial inhibition of prostaglandin and leukotriene synthesis in vitro, was investigated with respect to effects of the extract on the lipopolysaccharide (LPS) stimulated secretion of proinflammatory cytokines in human whole blood of healthy volunteers. In the assay system used, LPS stimulated human whole blood showed a straight increase of tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 beta (IL-1 beta) secretion reaching maximum concentrations within 24 hours following a plateau and slight decrease up to 65 hours, respectively. The concentrations of these cytokines was strongly positively correlated with the number of monocytes/macrophages of each volunteer. TNF-alpha and IL-1 beta concentration after LPS stimulation was significantly reduced by simultaneously given IDS 23 in a strictly dose dependent manner. At time 24 hours these cytokine concentrations were reduced by 50.8% and 99.7%, respectively, using the highest test IDS 23 assay concentration of 5 mg/ml ( $p < 0.001$ ). After 65 hours the corresponding inhibition was 38.9% and 99.9%, respectively ( $p < 0.001$ ). On the other hand IDS 23 showed no inhibition but stimulated IL-6 secretion in absence of LPS alone. Simultaneously given LPS and IDS 23 resulted in no further increase. In contrast to described effects on arachidonic acid cascade in vitro, tested *Urtica dioica* phenol carbon acid derivatives and flavonoids such as caffeic malic acid, caffeic acid, chlorogenic acid, quercetin and rutin did not influence LPS stimulated TNF-alpha, IL-1 beta and IL-6 secretion in tested concentrations up to  $5 \times 10^{-5}$  mol/l. These further findings on the pharmacological mechanism of action of *Urticae dioica* folia may explain the positive effects of this extract in the treatment of rheumatic diseases.

*Arzneimittelforschung.* 1996 Apr;46(4):389-94

### **Plant extracts from stinging nettle (*Urtica dioica*), an antirheumatic remedy, inhibit the proinflammatory transcription factor NF-kappaB.**

Activation of transcription factor NF-kappaB is elevated in several chronic inflammatory diseases and is responsible for the enhanced expression of many proinflammatory gene products. Extracts from leaves of stinging nettle (*Urtica dioica*) are used as antiinflammatory remedies in rheumatoid arthritis. Standardized preparations of these extracts (IDS23) suppress cytokine production, but their mode of action remains unclear. Here we demonstrate that treatment of different cells with IDS23 potently inhibits NF-kappaB activation. An inhibitory effect was observed in response to several stimuli, suggesting that IDS23 suppressed a common NF-kappaB pathway. Inhibition of NF-kappaB activation by IDS23 was not mediated by a direct modification of DNA binding, but rather by preventing degradation of its inhibitory subunit I-kappaB-alpha. Our results suggests that part of the antiinflammatory effect of *Urtica* extract may be ascribed to its inhibitory effect on NF-kappaB activation.

*FEBS Lett.* 1999 Jan 8;442(1):89-94

### **Effects of the antirheumatic remedy Hox alpha-a new stinging nettle leaf extract-on matrix metalloproteinases in human chondrocytes in vitro.**

Inflammatory joint diseases are characterized by enhanced extracellular matrix degradation which is predominantly mediated by cytokine-stimulated upregulation of matrix metalloproteinase (MMP) expression. Besides tumor necrosis factor-alpha (TNF-alpha), interleukin-1 beta (IL-1 beta) produced by articular chondrocytes and synovial macrophages, is the most important cytokine stimulating MMP expression under inflammatory conditions. Blockade of these two cytokines and their downstream

effectors are suitable molecular targets of antirheumatic therapy. Hox alpha is a novel stinging nettle (*Urtica dioica/Urtica urens*) leaf extract used for treatment of rheumatic diseases. The aim of the present study was to clarify the effects of Hox alpha and the monosubstance 13-HOTrE (13-Hydroxyoctadecatrienic acid) on the expression of matrix metalloproteinase-1, -3 and -9 proteins (MMP-1, -3, -9). Human chondrocytes were cultured on collagen type-II-coated petri dishes, exposed to IL-1beta and treated with or without Hox alpha and 13-HOTrE. A close analysis by immunofluorescence microscopy and western blot analysis showed that Hox alpha and 13-HOTrE significantly suppressed IL-1beta-induced expression of matrix metalloproteinase-1, -3 and -9 proteins on the chondrocytes in vitro. The potential of Hox alpha and 13-HOTrE to suppress the expression of matrix metalloproteinases may explain the clinical efficacy of stinging nettle leaf extracts in treatment of rheumatoid arthritis. These results suggest that the monosubstance 13-HOTrE is one of the more active antiinflammatory substances in Hox alpha and that Hox alpha may be a promising remedy for therapy of inflammatory joint diseases.

*Histol Histopathol.* 2002 Apr;17(2):477-85

### **Oral aspirin and ibuprofen increase cytokine-induced synthesis of IL-1 beta and of tumour necrosis factor-alpha ex vivo.**

We investigated the effect of oral aspirin and ibuprofen on the ex vivo synthesis of interleukin-1 alpha (IL-1 alpha), IL-1 beta, IL-2, IL-6, tumor necrosis factor-alpha (TNF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) by stimulated peripheral blood mononuclear cells (PBMC) from healthy volunteers. Seven volunteers took 325 mg of aspirin daily for 14 days. Three weeks after ending aspirin medication, ex vivo IL-1 beta and TNF synthesis induced by exogenous IL-1 alpha was elevated threefold compared to the pre-aspirin value ( $P = 0.01$  and  $P = 0.005$ , respectively). Using lipopolysaccharide (LPS) as a stimulus, no influence of oral aspirin was observed. The increase in cytokine synthesis did not parallel decreased synthesis of prostaglandin E2 (PGE2). Seven weeks after discontinuation of aspirin, cytokine and PGE-2 production returned to pre-aspirin levels. Another seven volunteers took 200 mg of ibuprofen daily for 12 days. Again, there was no effect on LPS- or *Staphylococcus epidermidis*-induced cytokine synthesis. However, IL-1 alpha-induced synthesis of IL-1 beta was elevated to a mean individual increase of 538% ( $P < 0.001$ ) and synthesis of TNF was elevated to 270% ( $P < 0.001$ ) at the end of ibuprofen medication and two weeks after discontinuation of ibuprofen. There were parallel increases in PGE2 and both returned to their pre-ibuprofen levels five weeks after stopping. Although inhibitors of cyclo-oxygenase blunt PGE2-mediated symptoms such as fever and pain, we conclude that short term use of either aspirin or ibuprofen results in a 'rebound' increase in cytokine-induced cytokine synthesis that is not observed in LPS-induced cytokines.

*Immunology.* 1996 Feb;87(2):264-70

### **Mechanism of 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid.**

The formation of 5-lipoxygenase (EC 1.13.11.34) products from endogenous substrate by intact rat neutrophilic granulocytes and from exogenous arachidonic acid by rat granulocyte 105,000 x g supernatants and affinity chromatography-purified human leukocyte 5-lipoxygenase was inhibited by acetyl-11-keto-beta-boswellic acid (IC50 values of 1.5 microM, 8 microM, and 16 microM respectively). With other pentacyclic triterpenes lacking the 11-keto function and/or the carboxyl function on ring A (e.g., amyirin and ursolic acid), no 5-lipoxygenase inhibition was observed. The presence of the noninhibitory pentacyclic triterpenes both in intact cells and in the cell-free system caused a concentration-dependent reversal of the 5-lipoxygenase inhibition by acetyl-11-keto-beta-boswellic acid, whereas the inhibitory actions of 5-lipoxygenase inhibitors from different chemical classes (MK-886, L-739,010, ZM-230,487 and nordihydroguaiaretic acid) were not modified. The inhibition by acetyl-11-keto-beta-boswellic acid and the antagonism by noninhibitory pentacyclic triterpenes were not due to nonspecific lipophilic interactions, because lipophilic four-ring compounds (cholesterol, cortisone and testosterone) neither inhibited the activity of the 5-lipoxygenase nor antagonized the inhibitory action of acetyl-11-keto-beta-boswellic acid. Therefore, we conclude that acetyl-11-keto-beta-boswellic acid acts directly on the 5-lipoxygenase enzyme at a selective site for pentacyclic triterpenes that is different from the arachidonate substrate binding site.

*Mol Pharmacol.* 1995 Jun;47(6):1212-6

### **Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids.**

The rhizomes of *Zingiber officinale* (ginger) and *Alpinia officinarum* contain potent inhibitors against prostaglandin biosynthesizing enzyme (PG synthetase). Gingerols and diarylheptanoids were identified as active compounds. Their possible mechanism of action which was deduced from the structures of active compounds indicated that the inhibitors would also be active against arachidonate 5-lipoxygenase, an enzyme of leukotriene (LT) biosynthesis. This was verified by testing their inhibitory effects on 5-lipoxygenase prepared from RBL-1 cells. A diarylheptanoid with catechol group was the most active compound against 5-lipoxygenase, while yakuchinone A was the most active against PG synthetase.

*Chem Pharm Bull (Tokyo).* 1992 Feb;40(2):387-91.

## ABSTRACTS

### Andropause

#### **Neuroendocrine aging in men. Andropause and somatopause.**

Aging is accompanied by gradual but progressive reductions in the secretion of testosterone and growth hormone in men, and by alterations in body composition and functional capacity that, to some degree, undo the effects of puberty. Preventing or reversing these changes with the use of trophic factors, including androgens, growth hormone and growth hormone secretagogues, is an appealing prospect, but documenting the effectiveness of these interventions and their benefits and risks has proven to be a difficult undertaking that is far from complete. Small-scale clinical studies have shown that it is practicable to boost growth hormone and IGF-1 levels for periods of up to 12 months, and testosterone for up to 36 months, to reverse at least some age-related changes in body composition. Information regarding the effects of these interventions on strength, exercise capacity and the ability to perform activities of daily living is still sparse, and additional reports from recently completed or currently ongoing clinical trials will not provide sufficient data to make firm conclusions. From the limited information currently available, androgen supplementation may be of benefit in some men aged more than 65 years, particularly in men with low serum testosterone levels (< 2 ng/mL). In this group, supplemental androgen therapy would be expected to increase lean body mass, bone mass, and possibly strength. In older men with testosterone levels between 2 and 3.5 ng/mL, some benefit might result from androgen supplementation, but it is not yet clear whether the benefits outweigh the risks. For men in this category, one might consider a 6- to 12-month trial of therapy after a full discussion and explicit consent, followed by a reassessment of the value of ongoing treatment. The even more limited data on growth hormone or growth hormone secretagogue interventions in aging do not support their general clinical use in healthy older men. Growth hormone is much more expensive than testosterone and is not covered by insurance for off-label uses. Patients who persistently seek a trial of therapy should be encouraged to enroll in a study if one is locally available. All of the growth hormone studies reported to date have focused, generally for reasons of safety, on healthy and robust groups of older subjects, men in whom the need for intervention is least compelling and in whom the functional effects of treatment may be the most difficult to observe. Phase II studies of intermediate size and duration examining prefrail groups of elderly who are at greater risk for functional loss and who stand to benefit the most from either preventive or restorative interventions are underway but are limited to the intermediate outcomes of body composition, strength and function. Trials designed to assess clinically relevant final outcomes, such as falls, fractures, and institutionalization, are of necessity large-scale, long-term and expensive. Support for larger phase III studies of growth hormone is unlikely to be forthcoming until the phase II studies are completed and show further promise. A multicenter clinical trial of testosterone is currently being planned under the joint sponsorship of the National Institute on Aging, the Veterans Health Administration, and industry, aimed at assessing the effects of testosterone on the risk for falls and fractures. The results of this trial and other large clinical trials should help to better define the balance of benefits and risks of trophic factor intervention in normal older men.

*Endocrinol Metab Clin North Am. 2001 Sep;30(3):647-69*

#### **Estrogen production and action.**

Estradiol production is most commonly thought of as an endocrine product of the ovary; however, there are many tissues that have the capacity to synthesize estrogens from androgen and to use estrogen in a paracrine or intracrine fashion. In addition, other organs such as the adipose tissue can contribute significantly to the circulating pool of estrogens. There is increasing evidence that in both men and women extraglandular production of C(18) steroids from C(19) precursors is important in normal physiology as well as in pathophysiologic states. The enzyme aromatase is found in a number of human tissues and cells, including ovarian granulosa cells, the placental syncytiotrophoblast, adipose and skin fibroblasts, bone, and the brain, and it locally catalyzes the conversion of C(19) steroids to estrogens. Aromatase expression in adipose tissue and possibly the skin primarily accounts for the extraglandular (peripheral) formation of estrogen and increases as a function of body weight and advancing age. Sufficient circulating levels of the biologically active estrogen estradiol can be produced as a result of extraglandular aromatization of androstenedione to estrone that is subsequently reduced to estradiol in peripheral tissues to cause uterine bleeding and endometrial hyperplasia and cancer in obese anovulatory or postmenopausal women. Extraglandular aromatase expression in adipose tissue and skin (via increasing circulating levels of estradiol) and bone (via increasing local estrogen concentrations) is of paramount importance in slowing the rate of postmenopausal bone loss. Moreover, excessive or inappropriate aromatase expression was demonstrated in adipose fibroblasts surrounding a breast carcinoma, endometriosis-derived stromal cells, and stromal cells in endometrial cancer, giving rise to increased local estrogen concentrations in these tissues. Whether systemically delivered or locally produced, elevated estrogen levels will promote the growth of these steroid-responsive tissues. Finally, local estrogen biosynthesis by aromatase activity in the brain may be important in the regulation of various cognitive and hypothalamic functions. The regulation of aromatase expression in human cells via alternatively used promoters, which can be activated or inhibited by various hormones, increases the complexity of estrogen biosynthesis in the human body. Aromatase expression is under the control of the classically located proximal promoter II in the ovary and a far distal promoter I.1 (40 kilobases upstream of the translation initiation site) in the placenta. In skin, the promoter is I.4. In adipose tissue, 2 other promoters (I.4 and I.3) located between I.1 and II are used in addition to the ovarian-type promoter II. In addition, promoter use in adipose fibroblasts switches between promoters II/I.3 and I.4 upon treatments of these cells with PGE(2) versus

glucocorticoids plus cytokines. Moreover, the presence of a carcinoma in breast adipose tissue also causes a switch of promoter use from I.4 to II.3. Thus there can be complex mechanisms that regulate the extraglandular production of estrogen in a tissue-specific and state-specific fashion.

*J Am Acad Dermatol. 2001 Sep;45(3 Suppl):S116-24*

### **Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging.**

The present data show a dramatic decline in the circulating levels of dehydroepiandrosterone (DHEA), DHEA-sulfate (DHEA-S), androst-5-ene-3 beta,17 beta-diol (5-diol), 5-diol-sulfate, 5-diol-fatty acid esters and androstenedione in both men and women between the ages of 20 to 80 years. In the 50- to 60-yr-old group, serum DHEA decreased by 74% and 70% from its peak values in 20- to 30-yr-old men and women, respectively. The serum concentrations of the conjugated metabolites of dihydrotestosterone (DHT), namely androsterone (ADT)-G, androstane-3 alpha,17 beta-diol (3 alpha-diol-G), androstane-3 beta,17 beta-diol (3 beta-diol-G), and ADT-sulfate are the most reliable parameters of the total androgen pool in both men and women, whereas serum testosterone and DHT can be used as markers of testicular secretion in men and interstitial ovarian secretion in women. The serum concentration of these various conjugated androgen metabolites decreased by 40.8% to 72.8% between the 20- to 30-year-old and 70- to 80-yr-old age groups in men and women, respectively, thus suggesting a parallel decrease in the total androgen pool with age. As estimated by measurement of the circulating levels of these conjugated metabolites of DHT, it is noteworthy that women produce approximately 66% of the total androgens found in men. In women, most of these androgens originate from the transformation of DHEA and DHEA-S into testosterone and DHT in peripheral intracrine tissues, whereas in men the testes and DHEA and DHEA-S provide approximately equal amounts of androgens at the age of 50 to 60 years. An additional potentially highly significant observation is that the majority of the marked decline in circulating adrenal C19 steroids and their resulting androgen metabolites takes place between the age groups of 20- to 30-yr olds and 50- to 60-yr-olds, with smaller changes are observed after the age of 60 yr.

*J Clin Endocrinol Metab. 1997 Aug;82(8):2396-402*

### **Measures of bioavailable serum testosterone and estradiol and their relationships with muscle strength, bone density and body composition in elderly men.**

In the present cross-sectional study of 403 independently living elderly men, we tested the hypothesis that the decreases in bone mass, body composition, and muscle strength with age are related to the fall in circulating endogenous testosterone (T) and estrogen concentrations. We compared various measures of the level of bioactive androgen and estrogen to which tissues are exposed. After exclusion of subjects with severe mobility problems and signs of dementia, 403 healthy men (age, 73 to 94 year) were randomly selected from a population-based sample. Total T (TT), free T (FT), estrone (E1), estradiol (E2) and sex hormone-binding globulin (SHBG) were determined by RIA. Levels of non-SHBG-bound T (non-SHBG-T), FT (calc-FT), the TT/SHBG ratio, non-SHBG-bound E2, and free E2 were calculated. Physical characteristics of aging included muscle strength measured using dynamometry, total body bone mineral density (BMD), hip BMD, and body composition, including lean mass and fat mass, measured by dual-energy x-ray absorptiometry. In this population of healthy elderly men, calc-FT, non-SHBG-T, E1, and E2 (total, free, and non-SHBG bound) decreased significantly with age. T (total and non-SHBG-T) was positively related with muscle strength and total body BMD (for non-SHBG-T, respectively,  $\beta = 1.93 \pm 0.52$ ,  $P < 0.001$  and  $\beta = 0.011 \pm 0.002$ ,  $P < 0.001$ ). An inverse association existed between T and fat mass ( $\beta = -0.53 \pm 0.15$ ,  $P < 0.001$ ). Non-SHBG-T and calc-FT were more strongly related to muscle strength, BMD, and fat mass than TT and were also significantly related to hip BMD. E1 and E2 were both positively, independently associated with BMD (for E2,  $\beta = 0.21 \pm 0.08$ ,  $P < 0.01$ ). Non-SHBG-bound E2 was slightly strongly related to BMD than total E2. The positive relation between T and BMD was independent of E2. E1 and E2 were not related with muscle strength or body composition. In summary, bioavailable T, E1, total E2, and bioavailable E2 all decrease with age in healthy old men. In this cross-sectional study in healthy elderly men, non-SHBG-bound T seems to be the best parameter for serum levels of bioactive T, which seems to play a direct role in the various physiological changes that occur during aging. A positive relation with muscle strength and BMD and a negative relation with fat mass was found. In addition, both serum E1 and E2 seem to play a role in the age-related bone loss in elderly men, although the cross-sectional nature of the study precludes a definitive conclusion. Non-SHBG-bound E2 seems to be the best parameter of serum bioactive E2 in describing its positive relation with BMD.

*J Clin Endocrinol Metab. 2000 Sep;85(9):3276-82*

### **Low levels of endogenous androgens increase the risk of atherosclerosis in elderly men: the Rotterdam study.**

In both men and women, circulating androgen levels decline with advancing age. Until now, results of several small studies on the relationship between endogenous androgen levels and atherosclerosis have been inconsistent. In the population-based Rotterdam Study, we investigated the association of levels of dehydroepiandrosterone sulfate (DHEAS) and total and bioavailable testosterone with aortic atherosclerosis among 1,032 nonsmoking men and women aged 55 years and over. Aortic atherosclerosis was assessed by radiographic detection of calcified deposits in the abdominal aorta, which have been shown to reflect intimal atherosclerosis. Relative to men with levels of total and bioavailable testosterone in the lowest tertile, men with levels of these hormones in the highest tertile had age-adjusted relative risks of 0.4 [95% confidence interval (CI), 0.2-0.9] and 0.2

(CI, 0.1-0.7), respectively, for the presence of severe aortic atherosclerosis. The corresponding relative risks for women were 3.7 (CI, 1.2-11.6) and 2.3 (CI, 0.7-7.8). Additional adjustment for cardiovascular disease risk factors did not materially affect the results in men, whereas in women the associations diluted. Men with levels of total and bioavailable testosterone in subsequent tertiles were also protected against progression of aortic atherosclerosis measured after 6.5 years (SD +/- 0.5 years) of follow-up (P for trend = 0.02). No clear association between levels of DHEAS and presence of severe aortic atherosclerosis was found, either in men or in women. In men, a protective effect of higher levels of DHEAS against progression of aortic atherosclerosis was suggested, but the corresponding test for trend did not reach statistical significance. In conclusion, we found an independent inverse association between levels of testosterone and aortic atherosclerosis in men. In women, positive associations between levels of testosterone and aortic atherosclerosis were largely due to adverse cardiovascular disease risk factors.

*J Clin Endocrinol Metab.* 2002 Aug;87(8):3632-9

## ABSTRACTS

### Andropause

#### **Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men.**

Circulating testosterone (T) levels have behavioral and neurological effects in both human and nonhuman species. Both T concentrations and neuropsychological function decrease substantially with age in men. The purpose of this prospective, longitudinal study was to investigate the relationships between age-associated decreases in endogenous serum T and free T concentrations and declines in neuropsychological performance. Participants were volunteers from the Baltimore Longitudinal Study of Aging, aged 50 to 91 years at baseline T assessment. Four hundred seven men were followed for an average of 10 years, with assessments of multiple cognitive domains and contemporaneous determination of serum total T, SHBG, and a free T index (FTI). We administered neuropsychological tests of verbal and visual memory, mental status, visuomotor scanning and attention, verbal knowledge/language, visuospatial ability and depressive symptomatology. Higher FTI was associated with better scores on visual and verbal memory, visuospatial functioning, and visuomotor scanning and a reduced rate of longitudinal decline in visual memory. Men classified as hypogonadal had significantly lower scores on measures of memory and visuospatial performance and a faster rate of decline in visual memory. No relations between total T or the FTI and measures of verbal knowledge, mental status or depressive symptoms were observed. These results suggest a possible beneficial relationship between circulating free T concentrations and specific domains of cognitive performance in older men.

*J Clin Endocrinol Metab.* 2002 Nov;87(11):5001-7

#### **Endogenous sex hormones and cognitive function in older men.**

The objective of this study was to determine whether endogenous sex hormone levels predict cognitive function in older men. Our study design was an exploratory analysis in a population-based cohort in Rancho Bernardo, California. The study participants were 547 community-dwelling men 59 to 89 years of age at baseline who were not using testosterone or estrogen therapy. Between 1984 and 1987, sera were collected for measurement of endogenous total and bioavailable testosterone and estradiol levels. Between 1988 and 1991, 12 standard neuropsychological instruments were administered, including two items from the Blessed Information-Memory-Concentration (BIMC) Test, three measures of retrieval from the Buschke-Fuld Selective Reminding Test, a category fluency test, immediate and delayed recall from the Visual Reproduction Test, the Mini-Mental State Examination with individual analysis of the Serial Sevens and the "World" Backwards components, and the Trail-Making Test Part B. In age- and education-adjusted analyses, men with higher levels of total and bioavailable estradiol had poorer scores on the BIMC Test and Mini-Mental State Examination. Men with higher levels of bioavailable testosterone had better scores on the BIMC Test and the Selective Reminding Test (long-term storage). Five associations were U-shaped: total testosterone and total and bioavailable estradiol with the BIMC Test; bioavailable testosterone with the "World" test; and total estradiol with the Trail-Making Test. All associations were relatively weak but independent of age, education, body mass index, alcohol use, cigarette smoking and depression. In these older men, low estradiol and high testosterone levels predicted better performance on several tests of cognitive function. Linear and nonlinear associations were also found, suggesting that an optimal level of sex hormones may exist for some cognitive functions.

*J Clin Endocrinol Metab.* 1999 Oct;84(10):3681-5

#### **Testosterone influences spatial cognition in older men.**

Testosterone plays a role in the organization of behavior during development. The authors examined whether testosterone could play a maintenance role in behavior as well. In a double-blind manner, verbal and visual memory, spatial cognition, motor speed, cognitive flexibility and mood in a group of healthy older men who were supplemented for three months with testosterone were assessed. The increase in testosterone levels to 150% of baseline levels resulted in a significant enhancement of spatial cognition, but no change in any other cognitive domain was found. Testosterone supplementation influenced the endogenous production of estradiol, and estradiol was found to have an inverse relationship to spatial cognitive performance. These results suggest that testosterone supplementation can modify spatial cognition in older men; however, it is likely that this occurs through testosterone's influence on estrogen.

*Behav Neurosci.* 1994 Apr;108(2):325-32

#### **Testosterone supplementation improves spatial and verbal memory in healthy older men.**

**OBJECTIVE:** To determine the relationship between exogenous testosterone administration and cognitive abilities in a population of healthy older men. **BACKGROUND:** Serum levels of total and bioavailable testosterone gradually decrease with age in men and are associated with reductions in muscle mass, osteoporosis, decreased sexual activity and changes in cognition. **METHODS:** Twenty-five healthy, community-dwelling volunteers, aged 50 to 80 years, completed a randomized, double-blind, placebo-controlled study. Participants received weekly intramuscular injections of either 100 mg testosterone enanthate or

placebo (saline) for six weeks. Cognitive evaluations were conducted at baseline, week three, and week six of treatment by use of a battery of neuropsychologic tests. RESULTS: Circulating total testosterone was raised an average of 130% from baseline at week three and 116% at week six in the treatment group. Because of aromatization of testosterone, estradiol increased an average of 77% at week three and 73% at week six in the treatment group. Significant improvements in cognition were observed for spatial memory (recall of a walking route), spatial ability (block construction) and verbal memory (recall of a short story) in older men treated with testosterone compared with baseline and the placebo group, although improvements were not evident for all measures. CONCLUSIONS: The results suggest that short-term testosterone administration enhances cognitive function in healthy older men. However, it remains unclear whether these improvements in cognition are attributable to increased testosterone or estradiol levels, or both. The potential role of testosterone vs its metabolites on cognition requires further research.

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### **Sex steroids modify working memory.**

In the last ten years, numerous mechanisms by which sex steroids modify cortical function have been described. For example, estrogen replacement improves verbal memory in women, and animal studies have shown effects of estrogen on hippocampal synaptogenesis and function. Little is known about sex steroid effects on other aspects of memory, such as frontal lobe-mediated working memory. We examined the relationships between working memory and sex steroid concentrations and whether sex steroid supplementation would modify age-related loss of working memory in older men and women. Before hormone supplementation, working memory, tested with the Subject Ordered Pointing Test (SOP), was worse in older subjects than younger subjects, and there was no evidence of gender differences at either age. Testosterone supplementation improved working memory in older men, but a similar enhancement of working memory was not found in older women supplemented with estrogen. In men, testosterone and estrogen effects were reciprocal-with better working memory related to a higher testosterone to estrogen ratio. These results suggest that sex steroids can modulate working memory in men and can act as modulators of cognition throughout life.

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### **Synergistic effects of estrogen with androgen on the prostate-effects of estrogen on the prostate of androgen-administered rats and 5-alpha-reductase activity.**

To clarify the effects of estrogen on the prostate of androgen-treated rats, we administered estradiol (E2; 0.01 mg/day) to testosterone (T; 1 mg/day)-treated Wistar rats under various conditions. There were three variable factors: the age of the rat, the untreated period after castration, and the presence of testes. Under all conditions, E2 stimulated the growth of the prostate in T-treated rats compared with growth in rats in treated with T only. To analyze the mechanism underlying this enhancement of prostate growth by estrogen, we measured 5-alpha-reductase activity and calculated its kinetic parameters, i.e., both Vmax and Km. The Vmax of the nuclear fraction was higher in E2-administered T-treated rats than in T only-treated rats. In contrast, the Vmax of the microsomal fraction was lower in E2-administered rats. Km values in the two groups showed no significant differences. Elevation of the nuclear fraction of 5-alpha-reductase activity could explain the synergistic effects of estrogen on the prostate growth of androgen-treated rats.

*Prostate. 1994 Oct;25(4):169-76*

### **Transdermal dihydrotestosterone treatment of 'andropause'.**

Male aging coincides on average with progressive impairment of testicular function. The most striking plasma changes are an increase in sex hormone binding globulin (SHBG) and a decrease in non SHBG-bound testosterone, which is the only testosterone subfraction effectively bioavailable for target tissues. In healthy subjects the bioavailable testosterone declines by approximately 1% per year between 40 and 70 years but a more pronounced decline has been observed in non-healthy groups, especially in high cardiovascular risks groups. Relative androgen deficiency is likely to have unfavourable consequences on muscle, adipose tissue, bone, haematopoiesis, fibrinolysis, insulin sensitivity, central nervous system, mood and sexual function and might be treated by an appropriate androgen supplementation. The potential risk for prostate has been the main reason for limiting indications of such treatment. Testosterone (T) and dihydrotestosterone (DHT) are two potent androgens which have opposite effects regarding aromatase activity, an enzyme present in prostate stroma and suspected to have a pathogenic influence through local oestradiol synthesis. T is the main substrate for aromatase and oestradiol synthesis while DHT is not aromatizable and, at sufficient concentration, decreases T and oestradiol levels. A 1.8 years survey of 37 men aged 55 to 70 years treated with daily percutaneous DHT treatment suggested that high plasma levels of DHT (> 8.5 nmol/l) effectively induced clinical benefits while slightly but significantly reducing prostate size. Early stages of prostate hypertrophy require synergic stimulation by both DHT and oestradiol, and suppressing oestradiol instead of DHT seems easier and better adapted to the specific situation of aged hypogonadic men.

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### **Antiestrogens and selective estrogen receptor modulators reduce prostate cancer risk.**

The development of chemoprevention strategies against prostate cancer would have the greatest overall impact both medically and economically against prostate cancer. Estrogens are required for prostate carcinogenesis. Estrogenic stimulation through estrogen receptor alpha in a milieu of decreasing androgens contributes significantly to the genesis of benign prostatic hyperplasia, prostate dysplasia and prostate cancer. The ability of antiestrogens and selective estrogen receptor modulators (SERMs) to delay and to suppress prostate carcinogenesis is supported by preclinical, clinical and epidemiological studies. SERMs have many features that make them attractive candidates for prostate cancer chemoprevention including their favorable safety profile and efficacy in preclinical prostate cancer models. The true clinical benefits of SERMs for chemoprevention to prevent prostate cancer, however, should continue to be investigated through human clinical trials. A phase IIb/III human clinical trial is currently evaluating safety and efficacy of toremifene, a SERM, in men who have high-grade prostatic intraepithelial neoplasia.

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### **Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta.**

The rat, mouse and human estrogen receptor (ER) exists as two subtypes, ER alpha and ER beta, which differ in the C-terminal ligand-binding domain and in the N-terminal transactivation domain. In this study, we investigated the estrogenic activity of environmental chemicals and phytoestrogens in competition binding assays with ER alpha or ER beta protein, and in a transient gene expression assay using cells in which an acute estrogenic response is created by cotransfecting cultures with recombinant human ER alpha or ER beta complementary DNA (cDNA) in the presence of an estrogen-dependent reporter plasmid. Saturation ligand-binding analysis of human ER alpha and ER beta protein revealed a single binding component for [<sup>3</sup>H]-17beta-estradiol (E2) with high affinity [dissociation constant (K<sub>d</sub>) = 0.05 - 0.1 nM]. All environmental estrogenic chemicals [polychlorinated hydroxybiphenyls, dichlorodiphenyltrichloroethane (DDT) and derivatives, alkylphenols, bisphenol A, methoxychlor and chlordecone] compete with E2 for binding to both ER subtypes with a similar preference and degree. In most instances the relative binding affinities (RBA) are at least 1000-fold lower than that of E2. Some phytoestrogens such as coumestrol, genistein, apigenin, naringenin and kaempferol compete stronger with E2 for binding to ER beta than to ER alpha. Estrogenic chemicals, as for instance nonylphenol, bisphenol A, o, p'-DDT and 2',4',6'-trichloro-4-biphenylol stimulate the transcriptional activity of ER alpha and ER beta at concentrations of 100-1000 nM. Phytoestrogens, including genistein, coumestrol and zearalenone stimulate the transcriptional activity of both ER subtypes at concentrations of 1-10 nM. The ranking of the estrogenic potency of phytoestrogens for both ER subtypes in the transactivation assay is different; that is, E2 >> zearalenone = coumestrol > genistein > daidzein > apigenin = phloretin > biochanin A = kaempferol = naringenin > formononetin = ipriflavone = quercetin = chrysin for ER alpha and E2 >> genistein = coumestrol > zearalenone > daidzein > biochanin A = apigenin = kaempferol = naringenin > phloretin = quercetin = ipriflavone = formononetin = chrysin for ER beta. Antiestrogenic activity of the phytoestrogens could not be detected, except for zearalenone which is a full agonist for ER alpha and a mixed agonist-antagonist for ER beta. In summary, while the estrogenic potency of industrial-derived estrogenic chemicals is very limited, the estrogenic potency of phytoestrogens is significant, especially for ER beta, and they may trigger many of the biological responses that are evoked by the physiological estrogens.

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### **Effects of heavy-resistance training on hormonal response patterns in younger vs. older men.**

To examine the adaptations of the endocrine system to heavy-resistance training in younger vs. older men, two groups of men (30 and 62 year old) participated in a 10-wk periodized strength-power training program. Blood was obtained before, immediately after, and 5, 15 and 30 min after exercise at rest before and after training and at rest at -3, 0, 6 and 10 wk for analysis of total testosterone, free testosterone, cortisol, growth hormone, lactate and ACTH analysis. Resting values for insulin-like growth factor (IGF)-I and IGF-binding protein-3 were determined before and after training. A heavy-resistance exercise test was used to evaluate the exercise-induced responses (four sets of 10-repetition maximum squats with 90 s of rest between sets). Squat strength and thigh muscle cross-sectional area increased for both groups. The younger group demonstrated higher total and free testosterone and IGF-I than the older men, training-induced increases in free testosterone at rest and with exercise, and increases in resting IGF-binding protein-3. With training the older group demonstrated a significant increase in total testosterone in response to exercise stress along with significant decreases in resting cortisol. These data indicate that older men do respond with an enhanced hormonal profile in the early phase of a resistance training program, but the response is different from that of younger men.

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