

LE Magazine January 2002

## ABSTRACTS

## I3C/DIM

Indole-3-carbinol is a negative regulator of estrogen receptor-alpha signaling in human tumor cells.

Estrogen, via its binding to the estrogen receptor (ER), plays an important role in breast cancer cell proliferation and tumor development. Indole-3-carbinol (I3C), a compound occurring naturally in cruciferous vegetables, exhibits a potent antitumor activity via its regulation of estrogen activity and metabolism. This study was designed to determine the effect of I3C on the potential to inhibit the ER-alpha. Using a reporter gene driven by the estrogen receptor, I3C (10-125 micromol/L) significantly repressed the 17 $\beta$ -estradiol (E2)-activated ER-alpha signaling in a dose-dependent manner. I3C and breast cancer susceptibility gene 1 (BRCA1) synergistically inhibited transcriptional activity of ER-alpha. Moreover, I3C down-regulated the expression of the estrogen-responsive genes, pS2 and cathepsin-D, and up-regulated BRCA1. The inhibitory effects of I3C did not contribute to its cytotoxic effects because these activities were observed at less than toxic concentrations. These results further suggest that antitumor activities of I3C are associated not only with its regulation of estrogen activity and metabolism, but also its modulation of ER transcription activity.

J Nutr 2000 Dec;130(12):2927-31

Oligomerization of indole-3-carbinol in aqueous acid.

Indole-3-carbinol [I3C, also called 3-(hydroxymethyl)indole] is a naturally occurring modulator of carcinogenesis with a biological activity that is at least partially dependent on its conversion to active substances in acidic media. We compared the identities of the major oligomeric products of I3C produced under conditions approximating those found in gastric juice with the reported identities of products of 3-substituted indoles produced under enzymatic and other nonenzymatic conditions. After a 10-min treatment in aqueous HCl solution, I3C was converted in 18% yield to a mixture of acetonitrile-soluble products, the major components of which (as determined by HPLC) were diindol-3-ylmethane (5.9%), 5,6,11,12,17,18-hexahydrocyclohept[1,2-b:4,5-b':7,8-b'']triiindole (2.0%), and [2-(indol-3-ylmethyl)indol-3-yl]indol-3-ylmethane (5.9%). Tentative assignments were made for 3,3-bis(indol-3-ylmethyl)indolenine (0.59%), a symmetrical cyclic tetramer (0.64%), and a linear tetramer (1.1%). Indolo[3,2-b]carbazole (ICZ) was formed slowly in aqueous acidic solutions in low yields (2.0 ppm) which increased to greater than 90 ppm following addition of an organic solvent [tetrahydrofuran (THF) or dimethylformamide (DMF)] to a neutralized solution. Relative yields of trimers vs dimer increased with decreasing pH and with decreasing starting concentration of I3C. Evidence is presented that ICZ formation may not involve radical intermediates as is characteristic of photodynamic processes. A mechanistic rationale is presented for the formation of the identified products.

Chem Res Toxicol 1992 Mar-Apr;5(2):188-93

Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, a major in vivo product of dietary indole-3-carbinol.

Under acidic conditions, indole-3-carbinol (I3C) is converted to a series of oligomeric products thought to be responsible for the biological effects of dietary I3C. Chromatographic separation of the crude acid mixture of I3C, guided by cell proliferation assay in human MCF-7 cells, resulted in the isolation of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane (LTr-1) as a major antiproliferative component. LTr-1 inhibited the growth of both estrogen-dependent (MCF-7) and -independent (MDA-MB-231) breast cancer cells by approximately 60% at a non-lethal concentration of 25 microM. LTr-1 had no apparent effect on the proliferation of MCF-7 cells in the absence of estrogen. LTr-1 was a weak ligand for the estrogen receptor (ER) (IC50 70 microM) and efficiently inhibited the estradiol (E2)-induced binding of the ER to its cognate DNA responsive element. The antagonist effects of LTr-1 also were exhibited in assays of endogenous pS2 gene expression and in cells transiently transfected with an estrogen-responsive reporter construct (pERE-vit-CAT). LTr-1 activated both binding of the aryl hydrocarbon (Ah) receptor to its cognate DNA responsive element and expression of the Ah receptor-responsive gene CYP1A1. LTr-1 was a competitive inhibitor of CYP1A1-dependent ethoxyresorufin-O-deethylase (EROD) activity. In summary, these results demonstrated that LTr-1, a major in vivo product of I3C, could inhibit the proliferation of both estrogen-dependent and -independent breast tumor cells and that LTr-1 is an antagonist of estrogen receptor function and a weak agonist of Ah receptor function.

Ligand-independent activation of estrogen receptor function by 3, 3'-diindolylmethane in human breast cancer cells.

3,3'-Diindolylmethane (DIM), a major in vivo product of acid-catalyzed oligomerization of indole-3-carbinol (I3C), is a promising anticancer agent present in vegetables of the Brassica genus. We investigated the effects of DIM on estrogen-regulated events in human breast cancer cells and found that DIM was a promoter-specific activator of estrogen receptor (ER) function in the absence of 17beta-estradiol (E(2)). DIM weakly inhibited the E(2)-induced proliferation of ER-containing MCF-7 cells and induced proliferation of these cells in the absence of steroid, by approximately 60% of the E(2) response. DIM had little effect on proliferation of ER-deficient MDA-MB-231 cells, suggesting that it is not generally toxic at these concentrations. Although DIM did not bind to the ER in this concentration range, as shown by a competitive ER binding assay, it activated the ER to a DNA-binding species. DIM increased the level of transcripts for the endogenous pS2 gene and activated the estrogen-responsive pERE-vit-CAT and pS2-tk-CAT reporter plasmids in transiently transfected MCF-7 cells. In contrast, DIM failed to activate transcription of the simple E(2)- and diethylstilbesterol-responsive reporter construct pATC2. The estrogen antagonist ICI 182780 (7alpha-[9-[(4,4,5,5,5-pentafluoropentyl)sulfonyl]nonyl]-estra-1,3,5(10)-triene-3, 17beta-diol) was effective against DIM-induced transcriptional activity of the pERE-vit-CAT reporter, which further supports the hypothesis that DIM is acting through the ER. We demonstrated that ligand-independent activation of the ER in MCF-7 cells could be produced following treatment with the D1 dopamine receptor agonist SKF-82958 [(+/-)6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4, 5-tetrahydro-1H-3-benzazepinehydrobromide]. We also demonstrated that the agonist effects of SKF-82958 and DIM, but not of E(2), could be blocked by co-treatment with the protein kinase A (PKA) inhibitor H-89 (N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide). These results have uncovered a promoter-specific, ligand-independent activation of ER signaling for DIM that may require activation by PKA, and suggest that this major I3C product may be a selective activator of ER function.

Biochem Pharmacol 2000 Jul 15;60(2):167-77

Aryl hydrocarbon receptor-mediated antiestrogenic and antitumorigenic activity of diindolylmethane.

Phytochemicals such as indole-3-carbinol (I3C) and sulforaphane are components of cruciferous vegetables which exhibit antitumorigenic activity associated with altered carcinogen metabolism and detoxification. Diindolylmethane (DIM) is a major acid-catalyzed metabolite of I3C formed in the gut that binds to the aryl hydrocarbon receptor (AhR) and treatment of MCF-7 human breast cancer cells with 10-50 microM DIM resulted in rapid formation of the nuclear AhR complex and induction of CYP1A1 gene expression was observed at concentrations >50 microM. Previous studies have demonstrated that 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), a high affinity AhR ligand, inhibits 17beta-estradiol (E2)-induced responses in MCF-7 cells and growth of E2-dependent 7,12-dimethylbenzanthracene (DMBA)-induced mammary tumors in female Sprague-Dawley rats. Results of this study show that like TCDD, DIM inhibits E2-induced proliferation of MCF-7 cells, reporter gene activity in cells transiently transfected with an E2-responsive plasmid (containing a frog vitellogenin A2 gene promoter insert) and down-regulates the nuclear estrogen receptor. Moreover, DIM (5 mg/kg every other day) also inhibits DMBA-induced mammary tumor growth in Sprague-Dawley rats and this was not accompanied by induction of hepatic CYP1A1-dependent activity. Thus, DIM represents a new class of relatively non-toxic AhR-based antiestrogens that inhibit E2-dependent tumor growth in rodents and current studies are focused on development of analogs for clinical treatment of breast cancer.

Carcinogenesis 1998 Sep;19(9):1631-9

Continued on Page 2 of 4

[Back to the Magazine Forum](#)

## ABSTRACTS

Distinct forms of hepatic androgen 6 beta-hydroxylase induced in the rat by indole-3-carbinol and pregnenolone carbonitrile.

The ability of indole-3-carbinol (IC), an anticarcinogen present in cruciferous vegetables, to induce CYP1A1, CYP1A2, CYP2B1/2, CYP2E1 and CYP3A1/2 in female rat liver was determined by Western analysis using monoclonal antibodies and compared to effects produced by pregnenolone carbonitrile in animals of both sexes. The ontogeny of induction of these cytochrome P450 isozymes in response to oral administration of IC was also investigated. An inverse correlation was observed between the 6 beta-hydroxylation of androsterone (A) and the induction by IC of CYP3A1/2, the P450 isozyme responsible for the bulk of hepatic 6 beta-hydroxylation of 4-androstenedione (AD). The effect of inhibitors on the formation of 6 beta-OHA from A or AD was also determined and shown to differ from their action on the P450 isozymes involved in the formation of the 6 beta-hydroxylated derivatives of AD or lithocholic acid. The results indicate that the enzyme induced by IC is distinct from the CYP3A1/2 which catalyzes hydroxylations at position 6 beta, allylic in AD but not in the fully saturated ring system of A. The increased hepatic conversion of A to its biologically less active 6 beta-OHA metabolite after treatment of female rats with IC could possibly contribute to the anticarcinogenic action of indole carbinols. It is also proposed that the action of multiple inducers present in cruciferous and other vegetables might produce androgen metabolic profiles very different from those produced by individual components isolated from them.

J Steroid Biochem Mol Biol 1994 Nov;51(3-4):219-25

Indole-3-carbinol inhibits the expression of cyclin-dependent kinase-6 and induces a G1 cell cycle arrest of human breast cancer cells independent of estrogen receptor signaling.

Indole-3-carbinol (I3C), a naturally occurring component of Brassica vegetables such as cabbage, broccoli, and Brussels sprouts, has been shown to reduce the incidence of spontaneous and carcinogen-induced mammary tumors. Treatment of cultured human MCF7 breast cancer cells with I3C reversibly suppresses the incorporation of [3H]thymidine without affecting cell viability or estrogen receptor (ER) responsiveness. Flow cytometry of propidium iodide-stained cells revealed that I3C induces a G1 cell cycle arrest. Concurrent with the I3C-induced growth inhibition, Northern blot and Western blot analyses demonstrated that I3C selectively abolished the expression of cyclin-dependent kinase 6 (CDK6) in a dose- and time-dependent manner. Furthermore, I3C inhibited the endogenous retinoblastoma protein phosphorylation and CDK6 phosphorylation of retinoblastoma in vitro to the same extent. After the MCF7 cells reached their maximal growth arrest, the levels of the p21 and p27 CDK inhibitors increased by 50%. The antiestrogen tamoxifen also suppressed MCF7 cell DNA synthesis but had no effect on CDK6 expression, while a combination of I3C and tamoxifen inhibited MCF7 cell growth more stringently than either agent alone. The I3C-mediated cell cycle arrest and repression of CDK6 production were also observed in estrogen receptor-deficient MDA-MB-231 human breast cancer cells, which demonstrates that this indole can suppress the growth of mammary tumor cells independent of estrogen receptor signaling. Thus, our observations have uncovered a previously undefined antiproliferative pathway for I3C that implicates CDK6 as a target for cell cycle control in human breast cancer cells. Moreover, our results establish for the first time that CDK6 gene expression can be inhibited in response to an extracellular antiproliferative signal.

J Biol Chem 1998 Feb 13;273(7):3838-47

2,3,7,8-Tetrachlorodibenzo-p-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells.

Diindolylmethane (DIM) is an acid-catalyzed condensation product of indole-3-carbinol, a constituent of cruciferous vegetables, and is formed in the stomach. DIM alters estrogen metabolism and inhibits carcinogen-induced mammary tumor growth in rodents. DIM is a weak agonist for the aryl hydrocarbon (Ah) receptor and blocks the effects of estrogens via inhibitory Ah receptor-estrogen receptor cross-talk. DIM and various structural analogs were examined in H295R cells for effects on 3 cytochrome P450 (CYP) enzymes involved in estrogen synthesis and/or metabolism: CYP1A1, CYP1B1, and CYP19 (aromatase). Aromatase activity was measured by conversion of 1 beta-(3)H-androstenedione to estrone and (3)H(2)O. H295R cells were exposed to the test chemicals dissolved in dimethyl sulfoxide for 24 h prior to analyses. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) (0--30 nM) and DIM (0--10 microM) induced ethoxyresorufin-O-deethylase (EROD) activity, as a measure of CYP1A1 and possibly 1B1 activity, with EC(50) values of about 0.3 nM and 3 microM, respectively. DIM, but not TCDD, induced aromatase activity with an apparently maximal 2-fold increase at 10 microM; higher concentrations of DIM and many of its analogs were cytotoxic. TCDD (30 nM) significantly increased CYP1A1 and 1B1 mRNA levels, but had no effect on mRNA for CYP19. DIM (3 microM) significantly increased mRNA levels for all three CYPs: DIM analogs with substitutions on the 5 and 5' position (3 microM) induced aromatase and EROD activity, together with mRNA levels of CYP1A1, 1B1, and 19; analogs that were substituted on the central carbon of the methane group showed little or no inductive activity toward the CYPs: In conclusion, DIM and several of its analogs appear to induce CYPs

via multiple yet distinct pathways in H295R human adrenocortical carcinoma cells.

Toxicol Sci 2001 May;61(1):40-8

Ah receptor binding properties of indole carbinols and induction of hepatic estradiol hydroxylation.

The effect of route of administration on the ability of indole-3-carbinol (I3C), an anticarcinogen present in cruciferous vegetables, to induce estradiol 2-hydroxylase (EH) in female rat liver microsomes was investigated and compared to that of its main gastric conversion product, 3,3'-diindolylmethane (DIM). This dimer was more potent than I3C after either oral or intraperitoneal administration and was also a better in vitro inhibitor of EH in control and I3C-induced hepatic microsomes. The induction of both CYP1A1 and 1A2 in about equal amounts by I3C and DIM as well as of CYP2B1/2 was demonstrated using monoclonal antibodies. DIM, isosafrole, beta-naphthoflavone, 3-methylcholanthrene and naringenin added in vitro inhibited EH strongly in induced microsomes but gestodene was a better inhibitor of estrogen 2-hydroxylation in liver microsomes from untreated female rats. The binding affinities of I3C and DIM to the Ah receptor were compared to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) by competition studies, and the IC50 values were shown to be  $2.0 \times 10^{-9}$  M,  $5.0 \times 10^{-5}$  M and  $2.3 \times 10^{-3}$  M for TCDD, DIM and I3C, respectively. The ability of I3C or DIM to cause in vitro transformation of the Ah receptor to a form able to bind to the dioxin-responsive element-3 (DRE3) was compared to that of TCDD and shown to parallel their abilities to compete for binding of [<sup>3</sup>H] TCDD to the Ah receptor. These experiments confirm and extend the proposals that dietary indoles induce specific cytochrome P450s in rat liver by a mechanism possibly involving the Ah receptor. The induced monooxygenases, in turn, increase the synthesis of 2-hydroxylated estrogens in the competing pathways of 2- and 16 alpha-hydroxylation which decreases the levels of 16 alpha-hydroxyestrone able to form stable covalent adducts with proteins including the estrogen receptor. Such steroid-protein interaction has been correlated with mammary carcinogenesis.

Biochem Pharmacol 1993 Mar 9;45(5):1129-36

Indole-3-carbinol (I3C) induced cell growth inhibition, G1 cell cycle arrest and apoptosis in prostate cancer cells.

Prostate cancer is one of the most common cancers in men and it is the second leading cause of cancer related death in men in the United States. Recent dietary and epidemiological studies have suggested the benefit of dietary intake of fruits and vegetables in lowering the incidence of prostate cancer. A diet rich in fruits and vegetables provides phytochemicals, particularly indole-3-carbinol (I3C), which may be responsible for the prevention of many types of cancer, including hormone-related cancers such as prostate. Studies to elucidate the role and the molecular mechanism(s) of action of I3C in prostate cancer, however, have not been conducted. In the current study, we investigated whether I3C had any effect against prostate cancer cells and, if so, attempts were made to identify the potential molecular mechanism(s) by which I3C elicits its biological effects on prostate cancer cells. Here we report for the first time that I3C inhibits the growth of PC-3 prostate cancer cells. Induction of G1 cell cycle arrest was also observed in PC-3 cells treated with I3C, which may be due to the observed effects of I3C in the up-regulation of p21(WAF1) and p27(Kip1) CDK inhibitors, followed by their association with cyclin D1 and E and down-regulation of CDK6 protein kinase levels and activity. The induction of p21(WAF1) appears to be transcriptionally upregulated and independent of the p53 responsive element. In addition, I3C inhibited the hyperphosphorylation of the Retinoblastoma (Rb) protein in PC-3 cells. Induction of apoptosis was also observed in this cell line when treated with I3C, as measured by DNA laddering and poly (ADP-ribose) polymerase (PARP) cleavage. We also found an up-regulation of Bax, and down-regulation of Bcl-2 in I3C-treated cells. These effects may also be mediated by the down-regulation of NF-kappaB observed in I3C treated PC-3 cells. From these results, we conclude that I3C inhibits the growth of PC-3 prostate cancer cells by inducing G1 cell cycle arrest leading to apoptosis, and regulates the expression of apoptosis-related genes. These findings suggest that I3C may be an effective chemopreventive or therapeutic agent against prostate cancer.

Oncogene 2001 May 24;20(23):2927-36

Continued on Page 3 of 4

[Back to the Magazine Forum](#)

## ABSTRACTS

### Whey

Oral supplementation with whey proteins increases plasma glutathione levels of HIV-infected patients.

HIV infection is characterized by an enhanced oxidant burden and a systemic deficiency of the tripeptide glutathione (GSH), a major antioxidant. The semi-essential amino acid cysteine is the main source of the free sulfhydryl group of GSH and limits its synthesis. Therefore, different strategies to supplement cysteine supply have been suggested to increase glutathione levels in HIV-infected individuals. The aim of this study was to evaluate the effect of oral supplementation with two different cysteine-rich whey protein formulas on plasma GSH levels and parameters of oxidative stress and immune status in HIV-infected patients. In a prospective double blind clinical trial, 30 patients (25 male, 5 female; mean age (+/- SD) 42 +/- 9.8 years) with stable HIV infection (221 +/- 102 CD4 + lymphocytes L-1) were randomized to a supplemental diet with a daily dose of 45 g whey proteins of either Protectamin (Fresenius Kabi, Bad Hamburg, Germany) or Immunocal (Immunotec, Vandreuil, Canada) for two weeks. Plasma concentrations of total, reduced and oxidized GSH, superoxide anion (O<sub>2</sub><sup>-</sup>) release by blood mononuclear cells, plasma levels of TNF-alpha and interleukins 2 and 12 were quantified with standard methods at baseline and after therapy. Pre-therapy, plasma GSH levels (Protectamin: 1.92 +/- 0.6 microM; Immunocal: 1.98 +/- 0.9 microM) were less than normal (2.64 +/- 0.7 microM, P = 0.03). Following two weeks of oral supplementation with whey proteins, plasma GSH levels increased in the Protectamin group by 44 +/- 56% (2.79 +/- 1.2 microM, P = 0.004) while the difference in the Immunocal group did not reach significance (+ 24.5 +/- 59%, 2.51 +/- 1.48 microM, P = 0.43). Spontaneous O<sub>2</sub><sup>-</sup> release by blood mononuclear cells was stable (20.1 +/- 14.2 vs. 22.6 +/- 16.1 nmol h<sup>-1</sup> 10<sup>6</sup> cells, P = 0.52) whereas PMA-induced O<sub>2</sub><sup>-</sup> release decreased in the Protectamin group (53.7 +/- 19 vs. 39.8 +/- 18 nmol h<sup>-1</sup> 10<sup>6</sup> cells, P = 0.04). Plasma concentrations of TNF-alpha and interleukins 2 and 12 (P > 0.08, all comparisons) as well as routine clinical parameters remained unchanged. Therapy was well tolerated. In glutathione-deficient patients with advanced HIV-infection, short-term oral supplementation with whey proteins increases plasma glutathione levels. A long-term clinical trial is clearly warranted to see if this "biochemical efficacy" of whey proteins translates into a more favourable course of the disease.

Eur J Clin Invest 2001 Feb;31(2):171-8

Lactokinins: whey protein-derived ACE inhibitory peptides.

Angiotensin-I-converting enzyme (ACE) has been classically associated with the renin-angiotensin system which regulates peripheral blood pressure. Peptides derived from the major whey proteins, i.e. alpha-lactalbumin (alpha-la) and beta-lactoglobulin (beta-lg) in addition to bovine serum albumin (BSA), inhibit ACE. Some of these inhibitory peptides, i.e. alpha-lactorphin (alpha-la f(50-53)), beta-lactorphin (beta-lg f(102-105)), beta-lactotensin (beta-lg f(146-149)) and albutensin A (BSA f(208-216)), have other bioactivities. The most potent lactokinins reported to date, (beta-lg f(142-148)), has an ACE IC<sub>50</sub> of 42.6 mumol/l. While they do not have the inhibitory potency of synthetic drugs commonly used in the treatment of hypertension, these naturally occurring peptides may represent nutraceutical/functional food ingredients for the prevention/treatment of high blood pressure. Studies with gastric and pancreatic proteinase digests of whey proteins indicate that enzyme specificity rather than extent of hydrolysis dictates the ACE inhibitory potency of whey hydrolysates.

Nahrung 1999 Jun;43(3):165-7

Nutritional therapy of chronic hepatitis by whey protein (non-heated).

In an open study the clinical efficacy of milk serum (whey) protein (Immunocal; cysteine content: 7.6-fold higher than that of casein) isolated from fresh milk and purified without heating was evaluated in 25 patients with chronic hepatitis B or C. Immunocal (12 g as protein) food (mousse) was given twice a day, in the morning and evening, for 12 weeks (test period). Casein (12 g as protein) food (mousse) was similarly given for two weeks prior to the start of the supplement with Immunocal food (induction period) and for four weeks after the end of the supplement with Immunocal food (follow-up period). Serum alanine aminotransferase (ALT) activity was reduced, and plasma glutathione (GSH) levels increased in six and five of eight patients with chronic hepatitis B, respectively, 12 weeks after the start of the supplement with Immunocal food. Serum lipid peroxide levels significantly decreased, and interleukin (IL)-2 levels and natural killer (NK) activity significantly increased. However, there were no significant Immunocal-related changes in 17 patients with chronic hepatitis C. These findings suggest that the long-term supplement with Immunocal alone may be effective for improving liver dysfunctions in patients with chronic hepatitis B.

J Med 2000;31(5-6):283-302

The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress.

**BACKGROUND:** Increased brain serotonin may improve the ability to cope with stress, whereas a decline in serotonin activity is involved in depressive mood. The uptake of the serotonin precursor, tryptophan, into the brain is dependent on nutrients that influence the cerebral availability of tryptophan via a change in the ratio of plasma tryptophan to the sum of the other large neutral amino acids (Trp-LNAA ratio). Therefore, a diet-induced increase in tryptophan availability may increase brain serotonin synthesis and improve coping and mood, particularly in stress-vulnerable subjects. **OBJECTIVE:** We tested whether alpha-lactalbumin, a whey protein with a high tryptophan content, may increase the plasma Trp-LNAA ratio and reduce depressive mood and cortisol concentrations in stress-vulnerable subjects under acute stress. **DESIGN:** Twenty-nine highly stress-vulnerable subjects and 29 relatively stress-invulnerable subjects participated in a double-blind, placebo-controlled study. Subjects were exposed to experimental stress after the intake of a diet enriched with either alpha-lactalbumin or sodium-caseinate. Diet-induced changes in the plasma Trp-LNAA ratio and prolactin were measured. Changes in mood, pulse rate, skin conductance, and cortisol concentrations were assessed before and after the stressor. **RESULTS:** The plasma Trp-LNAA ratio was 48% higher after the alpha-lactalbumin diet than after the casein diet ( $P = 0.0001$ ). In stress-vulnerable subjects this was accompanied by higher prolactin concentrations ( $P = 0.001$ ), a decrease in cortisol ( $P = 0.036$ ), and reduced depressive feelings ( $P = 0.007$ ) under stress. **CONCLUSIONS:** Consumption of a dietary protein enriched in tryptophan increased the plasma Trp-LNAA ratio and, in stress-vulnerable subjects, improved coping ability, probably through alterations in brain serotonin.

Am J Clin Nutr 2000 Jun;71(6):1536-44

Effect of supplementation with a cysteine donor on muscular performance.

Oxidative stress contributes to muscular fatigue. GSH is the major intracellular antioxidant, the biosynthesis of which is dependent on cysteine availability. We hypothesized that supplementation with a whey-based cysteine donor [Immunocal (HMS90)] designed to augment intracellular GSH would enhance performance. Twenty healthy young adults (10 men, 10 women) were studied presupplementation and 3 mo postsupplementation with either Immunocal (20 g/day) or casein placebo. Muscular performance was assessed by whole leg isokinetic cycle testing, measuring peak power and 30-s work capacity. Lymphocyte GSH was used as a marker of tissue GSH. There were no baseline differences (age, ht, wt, %ideal wt, peak power, 30-s work capacity). Follow-up data on 18 subjects (9 Immunocal, 9 placebo) were analyzed. Both peak power [ $13 \pm 3.5$  (SE) %,  $P < 0.02$ ] and 30-s work capacity ( $13 \pm 3.7\%$ ,  $P < 0.03$ ) increased significantly in the Immunocal group, with no change ( $2 \pm 9.0$  and  $1 \pm 9.3\%$ ) in the placebo group. Lymphocyte GSH also increased significantly in the Immunocal group ( $35.5 \pm 11.04\%$ ,  $P < 0.02$ ), with no change in the placebo group ( $-0.9 \pm 9.6\%$ ). This is the first study to demonstrate that prolonged supplementation with a product designed to augment antioxidant defenses resulted in improved volitional performance.

J Appl Physiol 1999 Oct;87(4):1381-5

New biological function of bovine alpha-lactalbumin: protective effect against ethanol- and stress-induced gastric mucosal injury in rats.

Although several studies have shown that milk protein components have a wide range of biological activities, the potential role of these proteins in the gastrointestinal mucosal defense system is less well elucidated. In this study, we investigated the effect of the major proteins in cow's milk on gastric mucosal injury by using two acute ulcer models in Wistar rats. Gastric mucosal injury was induced by either intragastric 60% ethanol-HCl or water-immersion restraint stress (23 degrees C, 7 h). Each test milk protein was orally administered 30 min before the induction of gastric injury. Among the major milk proteins, alpha-lactalbumin (alpha-LA) is demonstrated to have a marked protective effect against ethanol-induced gastric injury, with the same potency as that of the typical antiulcer agent, Selbex. Whey protein isolate (WPI), which contained 25% alpha-LA, also protected against gastric injury, while casein showed no effect. Comparative studies on the protective effect of the four major components of WPI, beta-lactoglobulin, alpha-LA, bovine serum albumin and gamma-globulins (immunoglobulins), on the basis of their contents in WPI revealed that alpha-LA was responsible for the protective effect of WPI, being about 4-fold more effective than WPI itself. Alpha-LA showed dose-dependent protection against gastric injury induced by stress as well as ethanol. Pretreatment with indomethacin (10 mg/kg body weight, s.c.), which is a potent inhibitor of endogenous prostaglandin synthesis, resulted in a significant reduction in the protective effect of alpha-LA. These results indicate that alpha-LA has marked antiulcer activity as an active component of cow's milk protein, and suggest that alpha-LA intake may serve to protect against gastric mucosal injury, in part through endogenous prostaglandin synthesis.

Biosci Biotechnol Biochem 2001 May;65(5):1104-11

[Back to the Magazine Forum](#)

## ABSTRACTS

Competition for glutathione precursors between the immune system and the skeletal muscle: pathogenesis of chronic fatigue syndrome.

The chronic fatigue syndrome (CFS) is typically associated or follows a recognized or presumed infection. Abnormalities of both humoral and cellular immunity have been demonstrated in a substantial proportion of patients with CFS. The most consistent findings are of impaired lymphocyte responses to mitogen. As an antioxidant, glutathione (GSH) is essential for allowing the lymphocyte to express its full potential without being hampered by oxiradical accumulation. Hence, protracted challenge of the immunocytes may lead to cellular GSH depletion. Because GSH is also essential to aerobic muscular contraction, an undesirable competition for GSH precursors between the immune and muscular systems may develop. It is conceivable that the priority of the immune system for the survival of the host has drawn to this vital area the ever-diminishing GSH precursors, thus depriving the skeletal muscle of adequate GSH precursors to sustain a normal aerobic metabolism resulting in fatigue and eventually myalgia.

Med Hypotheses 1999 Oct;53(4):347-9

Gelation of casein-whey mixtures: effects of heating whey proteins alone or in the presence of casein micelles.

The aim of the present work was to investigate the role of whey protein denaturation on the acid induced gelation of casein. This was studied by determining the effect of whey protein denaturation both in the presence and absence of casein micelles. The study showed that milk gelation kinetics and gel properties are greatly influenced by the heat treatment sequence. When the whey proteins are denatured separately and subsequently added to casein micelles, acid-induced gelation occurs more rapidly and leads to gels with a more particulated microstructure than gels made from co-heated systems. The gels resulting from heat-treatment of a mixture of pre-denatured whey protein with casein micelles are heterogeneous in nature due to particulates formed from casein micelles which are complexed with denatured whey proteins and also from separate whey protein aggregates. Whey proteins thus offer an opportunity not only to control casein gelation but also to control the level of syneresis, which can occur.

J Dairy Res 2001 Aug;68(3):471-81

Emulsion properties of casein and whey protein hydrolysates and the relation with other hydrolysate characteristics.

Casein and whey protein were hydrolyzed using 11 different commercially available enzyme preparations. Emulsion-forming ability and emulsion stability of the digests were measured as well as biochemical properties with the objective to study the relations between hydrolysate characteristics and emulsion properties. All whey protein hydrolysates formed emulsions with bimodal droplet size distributions, signifying poor emulsion-forming ability. Emulsion-forming ability of some casein hydrolysates was comparable to that of intact casein. Emulsion instability was caused by creaming and coalescence. Creaming occurred mainly in whey hydrolysate emulsions and in casein hydrolysate emulsions containing large emulsion droplets. Coalescence was dominant in casein emulsions with a broad particle size distribution. Emulsion instability due to coalescence was related to apparent molecular weight distribution of hydrolysates; a relative high amount of peptides larger than 2 kDa positively influences emulsion stability.

J Agric Food Chem 2001 Oct;49(10):5005-12

Hydrophobicity of whey protein concentrates measured by fluorescence quenching and its relation with surface functional properties.

Surface hydrophobicity of whey protein concentrate (WPC) under heated (85 degrees C for 5, 10, 20, 30, 40, and 60 min) and unheated conditions was measured using cis-parinaric acid (CPA), 1-anilino-8-naphthalenesulfonate (ANS), and a fluorescence quenching method using acrylamide as a quencher. This last method evaluates the degree of exposure of tryptophanyl residues in proteins to the solvent. The initial slope of Stern-Volmer plots,  $K(\text{app})$ , was used as an index of protein hydrophobicity. Surface hydrophobicity of WPC exhibited good relation with surface functional properties such as emulsifying and foaming. Analysis of the data obtained in this work showed that the fluorescence quenching method gave results similar to those obtained using CPA and ANS. Therefore, this simple technique is satisfactory in effectively obtaining information about the hydrophobicity of whey proteins.

J Agric Food Chem 2001 Oct;49(10):4784-9

Mechanical characterization of network formation during heat-induced gelation of whey protein dispersions.

The formation of gel network structures during isothermal heating of whey protein aqueous dispersions was probed by mechanical spectroscopy. It was anticipated that the pathway of the sol-to-gel transition of whey protein dispersions is quite different from that of ordinary cross-linking polymers (e.g., percolation-type transition), since aqueous solutions of native whey proteins have been shown to be highly structured even before gelation, in our previous study. At 20 degrees C, aqueous dispersions of beta-lactoglobulin, the major whey protein, and those of whey protein isolate (WPI), a mixture of whey proteins, exhibited solid-like mechanical spectra, i.e., the predominant storage modulus  $G'$  over the loss modulus  $G''$ , in a certain range of the frequency  $\omega$  (1-100 rad/s), regardless of the presence or absence of added NaCl. The existence of the added salt was, however, a critical factor for determining transitions in mechanical spectra during gelation at 70 degrees C. beta-Lactoglobulin dispersions in 0.1 mol/dm<sup>3</sup> NaCl maintained the solid-like nature during the entire gelation process and, after passing through the gelation point, satisfied parallel power laws ( $G' \approx G'' \approx \omega^n$ ) that have been proposed for a critical gel (i.e., the gel at the gelation point) that possesses a self-similar or fractal network structure. In contrast, beta-lactoglobulin dispersions without added salt exhibited a transition from solid-like [ $G'(\omega) > G''(\omega)$ ] to liquid-like [ $G'(\omega) < G''(\omega)$ ] mechanical spectra before gelation, but no parallel power law behavior was recognized at the gelation point. During extended heating time (aging), beta-lactoglobulin gels with 0.1 mol/dm<sup>3</sup> NaCl showed deviations from the parallel power laws, while spectra of gels without added NaCl approached the parallel power laws, suggesting that post-gelation reactions also significantly affect gel network structures. A percolation-type sol-to-gel transition was found only for WPI dispersions without added salt.

Biopolymers 2000;56(2):109-19

The effect of whey protein supplementation with and without creatine monohydrate combined with resistance training on lean tissue mass and muscle strength.

Our purpose was to assess muscular adaptations during 6 weeks of resistance training in 36 males randomly assigned to supplementation with whey protein (W; 1.2 g/kg/day), whey protein and creatine monohydrate (WC; 0.1 g/kg/day), or placebo (P; 1.2 g/kg/day maltodextrin). Measures included lean tissue mass by dual energy x-ray absorptiometry, bench press and squat strength (1-repetition maximum), and knee extension/flexion peak torque. Lean tissue mass increased to a greater extent with training in WC compared to the other groups, and in the W compared to the P group ( $p < .05$ ). Bench press strength increased to a greater extent for WC compared to W and P ( $p < .05$ ). Knee extension peak torque increased with training for WC and W ( $p < .05$ ), but not for P. All other measures increased to a similar extent across groups. Continued training without supplementation for an additional 6 weeks resulted in maintenance of strength and lean tissue mass in all groups. Males that supplemented with whey protein while resistance training demonstrated greater improvement in knee extension peak torque and lean tissue mass than males engaged in training alone. Males that supplemented with a combination of whey protein and creatine had greater increases in lean tissue mass and bench press than those who supplemented with only whey protein or placebo. However, not all strength measures were improved with supplementation, since subjects who supplemented with creatine and/or whey protein had similar increases in squat strength and knee flexion peak torque compared to subjects who received placebo.

Int J Sport Nutr Exerc Metab 2001 Sep;11(3):349-64

Solubility and moisture sorption isotherms of whey-protein-based edible films as influenced by lipid and plasticizer incorporation.

Plasticized whey-protein and whey-protein emulsion films were produced using sorbitol and glycerol as plasticizers and butterfat and candelilla wax as lipids. Protein, plasticizer, and lipid ratios were optimized to obtain acceptable free-standing flexible films. Water solubility (20 degrees C, 24 h) and moisture sorption isotherms (0.18-0.90 a(w), 25 degrees C) of the films were determined. The experimental moisture sorption isotherm values were fitted using the Guggenheim-Anderson-DeBoer (GAB) model. Solubility and equilibrium moisture contents (EMC) of the films were influenced by plasticizer and lipid incorporation. EMCs of all films increased rapidly at  $a(w) > \text{or} = 0.65$ . Incorporation of lipids reduced solubilities and EMCs of sorbitol- and glycerol-plasticized films. The effects of plasticizer and lipid type on GAB constants were also determined.

J Agric Food Chem 2001 Sep;49(9):4388-91

Affinity enrichment of bovine lactoferrin in whey.

Bovine lactoferrin was enriched in various whey samples by affinity chromatography using immobilized gangliosides. Bovine gangliosides were isolated from fresh buttermilk using a combination of ultrafiltration and organic extraction. Isolated gangliosides were covalently immobilized onto controlled-pore glass beads. The immobilized matrix contained 66 micrograms of gangliosides per gram of beads. After loading the matrix with reconstituted whey protein isolate (WPI) or whey protein concentrate (WPC), the matrix was washed with sodium phosphate buffer (pH 7) followed by sodium acetate buffer (pH 4) before elution of lactoferrin with 1 M NaCl in sodium acetate buffer. From the intensities of the protein bands in SDS-PAGE, lactoferrin constituted a minimum of 40% of the total protein in the salt eluted sample. WPI, pretreated by heating and ultrafiltration, showed the highest lactoferrin purity among protein sources, while WPI (10% wt/vol) showed the highest recovery. These results show that immobilized gangliosides can be used to enrich the lactoferrin content of whey.

[Back to the Magazine Forum](#)

All Contents Copyright © 1995-2009 Life Extension Foundation All rights reserved.

**LifeExtension®**

These statements have not been evaluated by the FDA. These products are not intended to diagnose, treat, cure or prevent any disease. The information provided on this site is for informational purposes only and is not intended as a substitute for advice from your physician or other health care professional or any information contained on or in any product label or packaging. You should not use the information on this site for diagnosis or treatment of any health problem or for prescription of any medication or other treatment. You should consult with a healthcare professional before starting any diet, exercise or supplementation program, before taking any medication, or if you have or suspect you might have a health problem. You should not stop taking any medication without first consulting your physician.