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1-EVALUATION OF SUPERVISED MULTIMODAL PREHABILITATION PROGRAMME IN CANCER PATIENTS UNDERGOING COLORECTAL RESECTION: A RANDOMIZED CONTROL TRIAL

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Abstract

BACKGROUND:

Prehabilitation has been previously shown to be more effective in enhancing postoperative functional capacity than rehabilitation alone. The purpose of this study was to determine whether a weekly supervised exercise session could provide further benefit to our current prehabilition program, when comparing to standard post-surgical rehabilitation.

METHODS:

A parallel-arm single-blind randomized control trial was conducted in patients scheduled for non-metastatic colorectal cancer resection. Patients were assigned to either a once weekly supervised prehabilitation (PREHAB+, n = 41) or standard rehabilitation (REHAB, n = 39) program. Both multimodal programs were home-based program and consisted of moderate intensity aerobic and resistance exercise, nutrition counseling with daily whey protein supplementation and anxiety-reduction strategies. Perioperative care was standardized for both groups as per enhanced recovery after surgery (ERAS®) guidelines. Functional exercise capacity, as determined by the 6-minute walk test distance (6MWD), was the primary outcome. Exercise quantity, intensity and energy expenditure was determined by the CHAMPS questionnaire.

RESULTS:

Both groups were comparable for baseline walking capacity (PREHAB+: 448 m [IQR 375-525] vs. REHAB: 461 m [419-556], p=.775) and included a similar proportion of patients who improved walking capacity (>20 m) during the preoperative period (PREHAB+: 54% vs. REHAB: 38%, p = .222). After surgery, changes in 6MWD were also similar in both groups. In PREHAB+, however, there was a significant association between physical activity energy expenditure and 6MWD (p < .01). Previously inactive patients were more likely to improve functional capacity due to PREHAB+ (OR 7.07 [95% CI 1.10-45.51]).

CONCLUSIONS:

The addition of a weekly supervised exercise session to our current prehabilitation program did not further enhance postoperative walking capacity when compared to standard REHAB care. Sedentary patients, however, seemed more likely to benefit from PREHAB+. An association was found between energy spent in physical activity and 6MWD. This information is important to consider when designing cost-effective prehabilitation programs.

CLIN NUTR. 2018 JUL 9. Pii:S0261-5614(18)31201-9.

2-TRIMODAL PREHABILITATION FOR COLORECTAL SURGERY ATTENUATES POST-SURGICAL LOSSES IN LEAN BODY MASS: A POOLED ANALYSIS OF RANDOMIZED CONTROLLED TRIALS.

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Abstract

BACKGROUND & AIMS:
Preservation of lean body mass is an important cancer care objective. The capacity for prehabilitation interventions to modulate the lean body mass (LBM) of colorectal cancer patients before and after surgery is unknown.

METHODS:
A pooled analysis of two randomized controlled trials of trimodal prehabilitation vs. trimodal rehabilitation at a single university-affiliated tertiary center employing Enhanced Recovery After Surgery (ERAS) care was conducted. The prehabilitation interventions included exercise, nutrition, and anxiety-reduction elements that began approximately four weeks before surgery and continued for eight weeks after surgery. The rehabilitation interventions were identical to the prehabilitation interventions but were initiated only after surgery. Body composition, measured using multifrequency bioelectrical impedance analysis, was recorded at baseline, pre-surgery, 4 and 8 weeks after surgery. The primary outcome was change in LBM before and after colorectal surgery for cancer. A mixed effects regression model was used to estimate changes in body mass and body composition over time controlling for age, sex, baseline body mass index (BMI), baseline six-minute walk test (6MWT), and postoperative compliance to the interventions. NCT02586701 &NCT01356264.

RESULTS:
Pooled data included 76 patients who followed prehabilitation and 63 patients who followed rehabilitation (n = 139). Neither group experienced changes in preoperative LBM. Compared to rehabilitated patients, prehabilitated patients had significantly more absolute and relative LBM at four and eight-weeks post-surgery in models controlling for age, sex, baseline BMI, baseline 6MWT, and compliance to the postoperative intervention.

CONCLUSION:
Trimodal prehabilitation attenuated the post-surgical LBM loss compared to the loss observed in patients who received the rehabilitation intervention. Patients who receive neither intervention (i.e., standard of care) would be likely to lose more LBM. Offering a prehabilitation program to colorectal cancer patients awaiting resection is a useful strategy to mitigate the impact of the surgical stress response on lean tissue in an ERAS setting, and, in turn, might have a positive impact on the cancer care course.

CLINICAL TRIAL REGISTRATION:
NCT02586701 &NCT01356264 (clinicaltrials.gov).

KEYWORDS:
Body composition; ERAS; LBM; Prehab; Preoperative; Surgical preparation

FREE RADIC BIOL MED. 2018 AUG 20;124:328-341

3-THE CYSTEINE-RICH WHEY PROTEIN SUPPLEMENT, IMMUNOCAL®, PRESERVES BRAIN GLUTATHIONE AND IMPROVES COGNITIVE, MOTOR, AND HISTOPATHOLOGICAL INDICES OF TRAUMATIC BRAIN INJUURY IN A MOUSE MODEL OF CONTROLLED CORTICAL IMPACT.

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Traumatic brain injury (TBI) is a major public health problem estimated to affect nearly 1.7 million people in the United States annually. Due to the often debilitating effects of TBI, novel preventative agents are highly desirable for at risk populations. Here, we tested a whey protein supplement, Immunocal®, for its potential to enhance resilience to TBI. Immunocal® is a non-denatured whey protein preparation which has been shown to act as a cysteine delivery system to increase levels of the essential antioxidant glutathione (GSH). Twice daily oral supplementation of CD1 mice with Immunocal® for 28 days prior to receiving a moderate TBI prevented an ~25% reduction in brain GSH/GSSG observed in untreated TBI mice. Immunocal® had no significant effect on the primary mechanical injury induced by TBI, as assessed by MRI, changes in Tau phosphorylation, and righting reflex time or apnea. However, pre-injury supplementation with Immunocal® resulted in statistically significant improvements in motor function (beam walk and rotarod) and cognitive function (Barnes maze). We also observed a significant preservation of corpus callosum width (axonal myelination), a significant decrease in degenerating neurons, a reduction in Iba1 (microglial marker), decreased lipid peroxidation, and preservation of brain-derived neurotrophic factor (BDNF) in the brains of Immunocal®-pretreated mice compared to untreated TBI mice. Taken together, these data indicate that pre-injury supplementation with Immunocal® significantly enhances the resilience to TBI induced by a moderate closed head injury in mice. We conclude that Immunocal® may hold significant promise as a preventative agent for TBI, particularly in certain high risk populations such as athletes and military personnel.

KEYWORDS: Cognitive function; Glutathione; Motor function; Neuroprotection; Traumatic brain injury

JAMA SURG. 2018 SEPT 5 DOI:10.1001/JAMASURG.2018. 1645

4-EFFECT OF EXERCISE AND NUTRITION PREHABILITATION ON FUNCTIONAL CAPACITY IN ESOPHAGOGASTRIC CANCER SURGERY. A RANDOMIZED CLINICAL TRIAL

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Abstract

Importance: Preserving functional capacity is a key element in the care continuum for patients with esophagogastric cancer. Prehabilitation, a preoperative conditioning intervention aiming to optimize physical status, has not been tested in upper gastrointestinal surgery to date.

Objective: To investigate whether prehabilitation is effective in improving functional status in patients undergoing esophagogastric cancer resection.

Design, Setting, and Participants: A randomized clinical trial (available-case analysis based on completed assessments) was conducted at McGill University Health Centre (Montreal, Quebec, Canada) comparing prehabilitation with a control group. Intervention consisted of preoperative exercise and nutrition optimization. Participants were adults awaiting elective esophagogastric resection for cancer. The study dates were February 13, 2013, to February 10, 2017.

Main Outcomes and Measures: The primary outcome was change in functional capacity, measured with absolute change in 6-minute walk distance (6MWD). Preoperative (end of the prehabilitation period) and postoperative (from 4 to 8 weeks after surgery) data were compared between groups.

Results: Sixty-eight patients were randomized, and 51 were included in the primary analysis. The control group were a mean (SD) age, 68.0 (11.6) years and 20 (80%) men. Patients in the prehabilitation group were a mean (SD) age, 67.3 (7.4) years and 18 (69%) men. Compared with the control group, the prehabilitation group had improved functional capacity both before surgery (mean [SD] 6MWD change, 36.9 [51.4] vs −22.8 [52.5] m; $P < .001$) and after surgery (mean [SD] 6MWD change, 15.4 [65.6] vs −81.8 [87.0] m; $P < .001$).

Conclusions and Relevance: Prehabilitation improves perioperative functional capacity in esophagogastric surgery. Keeping patients from physical and nutritional status decline could have a significant effect on the cancer care continuum.

Trial Registration: ClinicalTrials.gov Identifier: NCT01666158

SUPPORT CARE CANCER. 2017 Jan;25(1):33-40.

5-FOUR-WEEK PREHABILITATION PROGRAM IS SUFFICIENT TO MODIFY EXERCISE BEHAVIORS AND IMPROVE PREOPERATIVE FUNCTIONAL WALKING CAPACITY IN PATIENTS WITH COLORECTAL CANCER

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Abstract

PURPOSE:

High complication rates following colorectal surgery render many patients unable to fully regain functional capacity, thus seriously compromising quality of life. The aim of this study was to assess whether a 4-week trimodal prehabilitation program (exercise, nutritional supplementation, and counseling on relaxation techniques), implemented during the preoperative period, is sufficient to modify exercise behaviors and improve functional capacity of elderly patients scheduled for colorectal cancer surgery.

METHODS:

Patients were assigned to either a prehabilitation (PREHAB; n = 57) or matched time control group (CTRL; n = 59). Over the 4-week period prior to surgery, patients in PREHAB participated in a trimodal prehabilitation program. Patients in CTRL received the same program but only postoperatively. The Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire was used to measure physical activity levels, while the 6-min walk test (6MWT) was used for assessment of functional walking capacity. Measurements were collected at baseline and at the time of surgery.

RESULTS:

Over the preoperative period, patients in PREHAB significantly increased the amount of moderate- and vigorous-intensity physical activities that they performed. PREHAB patients also demonstrated a greater improvement in 6MWT compared to CTRL. At the time of surgery, a greater proportion of patients in PREHAB met current physical activity guidelines, as compared to CTRL.

CONCLUSIONS:

These findings highlight the positive effects of a trimodal prehabilitation program on patients' physical activity levels and functional walking capacity and demonstrate that modifying exercise behaviors and improving physical function within the 4-week preoperative period are an achievable goal.

KEYWORDS:

Cancer; Exercise; Functional capacity; Oncology; Prehabilitation;Rehabilitation

ACTA ONCOL. 2017 FEB;56(2):295-300.

6-MULTIMODAL PREHABILITATION IMPROVES FUNCTIONAL CAPACITY BEFORE AND AFTER COLORECTAL SURGERY FOR CANCER: A FIVE-YEAR RESEARCH EXPERIENCE.

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Abstract

BACKGROUND:

Multimodal prehabilitation is a preoperative conditioning intervention in form of exercise, nutritional assessment, whey protein supplementation, and anxiety-coping technique. Despite recent evidence suggesting that prehabilitation could improve functional capacity in patients undergoing colorectal surgery for cancer, all studies were characterized by a relatively small sample size. The aim of this study was to confirm what was previously found in three small population trials.

MATERIAL AND METHODS:

Data of 185 participants enrolled in a pilot single group study and two randomized control trials conducted at the McGill University Health Center from 2010 to 2015 were reanalyzed. Subjects performing trimodal prehabilitation (exercise, nutrition, and coping strategies for anxiety) were compared to the patients who underwent the trimodal program only after surgery (rehabilitation/control group). Functional capacity was assessed with the six-minute walk test (6MWT), a measure of the distance walked over six minutes (6MWD). A significant functional improvement was defined as an increase in 6MWD from baseline by at least 19 m. Changes in 6MWD before surgery, at four and eight weeks were compared between groups.

RESULTS:

Of the total study population, 113 subjects (61%) underwent prehabilitation. Changes in 6MWD in the prehabilitation group were higher compared to the rehabilitation/control group during the preoperative period {30.0 [standard deviation (SD) 46.7] m vs. -5.8 (SD 40.1) m, $p < 0.001$}, at four weeks [-11.2 (SD 72) m vs. -72.5 (SD 129) m, $p < 0.01$], and at eight weeks [17.0 (SD 84.0) m vs. -8.8 (SD 74.0) m, $p = 0.047$]. The proportion of subjects experiencing a significant preoperative improvement in physical fitness was higher in those patients who underwent prehabilitation [68 (60%) vs. 15 (21%), $p < 0.001$].

CONCLUSION:

In large secondary analysis, multimodal prehabilitation resulted in greater improvement in walking capacity throughout the whole perioperative period when compared to rehabilitation started after surgery.

FREE RADIC BIOL MED. 2017 SEP;110:162-175.

7-CYSTEINE-RICH WHEY PROTEIN ISOLATE (IMMUNOCAL®) AMELIORATES DEFICITS IN THE GFAP.HMOX1 MOUSE MODEL OF SCHIZOPHRENIA

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Abstract: Schizophrenia is a neuropsychiatric disorder that features neural oxidative stress and glutathione (GSH) deficits. Oxidative stress is augmented in brain tissue of GFAP.HMOX1 transgenic mice which exhibit schizophrenia-relevant characteristics. They whey protein isolate, Immunocal® serves as a GSH precursor upon oral administration. In this study, we treated GFAP.HMOX1 transgenic mice daily with either Immunocal (33mg/ml drinking water) or equivalent concentrations of casein (control) between the ages of 5 and 6.5 months. Immunocal attenuated many of the behavioral neurochemical and redox abnormalities observed in GFAP.HMOX1 mice. In addition to restoring GSH homeostasis in the CNS of the transgenic mice, the whey protein isolate augmented GSH reserves in the brains of wild-type animals. These results demonstrate that consumption of whey protein isolate augments GSH stores and antioxidant defenses in the healthy and diseased mammalian brain. Whey protein isolate supplementation (Immunocal) may constitute a safe and effective modality for the management of schizophrenia, an unmet clinical imperative.

KEYWORDS:
Astrocyte; Dopamine; Glutathione; Heme oxygenase-1; Immunocal; Neurodegenerative disorder; Neurodevelopmental disorder; Schizophrenia; Whey protein isolate

OXID MED CELL LONGEV. 2017:3103272 DOI.10. 1155.

8-A CYSTEINE-RICH WHEY SUPPLEMENT (IMMUNOCAL®) PROVIDES NEUROPROTECTION FROM DIVERSE OXIDATIVE STRESS-INDUCING AGENTS *IN VITRO* BY PRESERVING CELLULAR GLUTATHIONE

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Abstract: Oxidative stress is a principal mechanism underlying the pathophysiology of neurodegeneration. Therefore, nutritional enhancement of endogenous antioxidant defenses may represent a viable treatment option. We investigated the neuroprotective properties of a unique whey protein supplement (Immunocal®) that provides an essential precursor (cystine) for synthesis of the endogenous antioxidant, glutathione (GSH). Primary cultures of rat cerebellar granule neurons (CGNs), NSC34 motor neuronal cells, or HT22 hippocampal cells were preincubated in medium containing Immunocal and then subsequently treated with agents know to induce oxidative stress. Immunocal protected CGNs against neurotoxicity induced by the Bcl-2 inhibitor, HA14-1, the nitric oxide donor, sodium nitroprusside, $CuCl_2$, and $A1C1_3$. Immunocal also significantly reduced NSC34 cell death due to either H_2O_2 or glutamate and mitigated toxicity in HT22 cells overexpressing

β-amyloid $_{1-42}$. The neuroprotective effects of Immunocal were blocked by inhibition of γ-glutamyl-cysteine ligase, demonstrating dependence on de novo GSH synthesis. These findings indicate that sustaining GSH with Immunocal significantly protects neurons against diverse inducers of oxidative stress. Thus, Immunocal is a nutritional supplement worthy of testing in preclinical animal models of neurodegeneration and in future clinical trials of patients afflicted by these diseases.

JOURNAL OF THE NEUROLOGICAL SCIENCES 367 (2016) 162-170

9-BIOCHEMICAL AND CLINICAL EFFECTS OF WHEY PROTEIN SUPPLEMENTATION IN PARKINSON'S DISEASE: A PILOT STUDY.

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Abstract: Background: Parkinson's Disease (PD) is an oxidative stress-mediated degenerative disorder. Elevated plasma homocysteine (Hcy) is frequently found in the levodopa-treated PD patients, is associated with disease progression and is a marker of oxidative stress. Whey protein is a rich source of cysteine, and branched-chain amino acids (BCAA). It has been shown that supplementation with Whey protein increases glutathione synthesis and muscle strength.

Objectives & Methods: In this study, we conducted a placebo-controlled, double-blind study (NCTO1662414) to investigate the effects of undenatured Whey protein isolate supplementation for 6 months on plasma glutathione, plasma amino acids, and plasma Hcy in PD patients. Clinical outcome assessments included the unified Parkinson's disease rating scale (UPDRS) and striatal L-3,4-dihydroxy-6-(18)F-fluorophenylalanine (FDOPA) uptake were determined before and after supplementation. 15 patients received Whey protein, and 17 received Soy protein, served as a control group.

Results: Significant increases in plasma concentration of reduced glutathione and the ratio of reduced to oxidized glutathione were found in the Whey-supplemented patients but not in a control group. This was associated with a significant decrease of plasma levels of Hcy. The plasma levels of total glutathione were not significantly changed in either group. Plasma BCAA and essential amino acids (EAA) were significantly increased in the Whey-supplemented group only. The UPDRS and striatal FDOPA uptake in PD patients were not significantly ameliorated in either group. However, significant negative correlation was observed between the UPDRS and plasma BCAA and EAA in the pre-supplemented PD patients.

Conclusion: This study is the first to report that Whey protein supplementation significantly increases plasma reduced glutathione, the reduced to oxidized glutathione ratio, BCAAs and EAAs in patients with PD, together with a concomitant significant reduction of plasma Hcy. However, there were no significant changes in clinical outcomes. Long-term, large randomized clinical studies are needed to explore the benefits of Whey protein supplementation in the management of PD patients.

JOURNAL OF THE ACADEMY OF NUTRITION AND DIETETICS 2015; ARTICLE IN PRESS

10-PREHABILITATION WITH WHEY PROTEIN SUPPLEMENTATION ON PERIOPERATIVE FUNCTIONAL EXERCISE CAPACITY IN PATIENTS UNDERGOING COLORECTAL RESECTION FOR CANCER: A PILOT DOUBLE-BLINDED RANDOMIZED PLACEBO-CONTROLLED TRIAL.

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Abstract: Background: A previous comprehensive prehabilitation program, providing nutrition counseling with whey protein supplementation, exercise, and psychological care, initiated 4 weeks before colorectal surgery for cancer, improved functional capacity before surgery and accelerated functional recovery. Those receiving standard of care deteriorated. The specific role of nutritional prehabilitation alone on functional recovery is unknown.

Objective: This study was undertaken to estimate the impact of nutrition counseling with whey protein on preoperative functional walking capacity and recovery in patients undergoing colorectal resection for cancer.

Design: We conducted a double-blinded randomized controlled trial at a single university-affiliated tertiary center located in Montreal, Quebec, Canada. Colon cancer patients (n=48) awaiting elective surgery for nonmetastatic disease were randomized to receive either individualized nutrition counseling with whey protein supplementation to meet protein needs or individualized nutrition counseling with a nonnutritive placebo. Counseling and supplementation began 4 weeks before surgery and continued for 4 weeks after surgery.

Main Outcome Measure: The primary outcome was change in functional walking capacity as measured with the 6-minute walk test. The distance was recorded at baseline, the day of surgery, and 4 weeks after surgery. A change of 20 m was considered clinically meaningful.

Results: The whey group experienced a mean improvement in functional walking capacity before surgery of +20.8m, with a standard deviation of 42.6m and the placebo group improved by +1.2 (65.5) m (P=0.27). Four weeks after surgery, recovery rates were similar between groups (P=0.81).

Conclusion: Clinically meaningful improvements in functional walking capacity were achieved before surgery with whey protein supplementation. These pilot results are encouraging and justify larger-scale trials to define the specific role of nutrition prehabilitation on functional recovery after surgery.

J NUTR HEALTH AGING 2015;19(5):531-6

11-EFFECT OF CYSTEINE-RICH WHEY PROTEIN (IMMUNOCAL®) SUPPLEMENTATION IN COMBINATION WITH RESISTANCE TRAINING ON MUSCLE STRENGTH AND LEAN BODY MASS IN NON-FRAIL ELDERLY SUBJECTS: A RANDOMIZED, DOUBLE-BLIND CONTROLLED STUDY

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Abstract: *Objectives:* The purpose of the present study was to examine the effect of a cysteine-rich whey protein (Immunocal®) supplementation in combination with resistance training on muscle strength and lean body mass (LBM) in elderly individuals. We hypothesized that the cysteine-rich whey protein (Immunocal®) group would experience a greater increase in muscle strength and lean body mass versus the control group (casein). *Design:* Randomized double-blind controlled intervention study. *Setting:* Institut de Recherches Cliniques de Montréal in Montréal, Canada. *Participants:* Ninety-nine non-frail elderly subjects were recruited. *Intervention:* Participants were randomly assigned into two groups. The experimental group received a cysteine-rich whey protein isolate (Immunocal®) (20g/day) and the control group received casein (20g/day) during a 135-day period. In addition, both groups performed the same resistance training program (3 times per week). *Measurements:* Body composition (DXA) and muscle strength (leg press) were measured. *Results:* Of the 99 recruited participants, 84 completed the 135-day study period. Of these, 67 subjects (33 in the casein group and 34 in the Immunocal® group) complied and used at least 80% of the study product and completed at least 80% of their training sessions. Results in this selected group show an increase in all three muscle strength variables (absolute, normalized by BW and by LBM) by 31.0%, 30.9% and 30.0%, respectively in the casein group as well as 39.3%, 39.9% and 43.3% respectively in the Immunocal® group after the intervention ($p<0.05$). The increases in muscle strength favored Immunocal® versus casein by approximately 10% when expressed in kg per kg BW and in kg per kg LBM ($p< 0.05$) No significant changes were found between pre-and-post intervention in both groups for total LBM. *Conclusions:* Our findings showed increases in muscle strength in both groups after resistance training, however, significant additional increases were observed in muscle strength with the addition of a cysteine-rich whey protein (Immunocal®) versus casein.

TRIAL REGISTRATION:
ClinicalTrials.gov NCT00935610.

ANESTHESIOLOGY 2014; 121:937-47

12-PREHABILITATION VERSUS REHABILITATION A RANDOMIZED CONTROL TRIAL IN PATIENTS UNDERGOING COLORECTAL RESECTION FOR CANCER

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Abstract: *Background:* The preoperative period (prehabilitation) may represent a more appropriate time than the postoperative period to implement an intervention. The impact of prehabilitation on recovery of functional exercise capacity was thus studied in patients undergoing colorectal resection for cancer.

Methods: A parallel-arm single-blind superiority randomized controlled trial was conducted. Seventy-seven patients were randomized to receive either prehabilitation (n=38) or rehabilitation (n = 39). Both groups received a home-based intervention of moderate aerobic and resistance exercises, nutritional counseling with protein supplementation, and relaxation exercises initiated either 4 weeks before surgery (prehabilitation) or immediately after surgery (rehabilitation), and continued for 8 weeks after surgery. Patients were managed with an enhanced recovery pathway. Primary outcome was functional exercise capacity measured using the validated 6-min walk test.

Results: Median duration of prehabilitation was 24.5 days. While awaiting surgery, functional walking capacity increased (≥20m) in a higher proportion of the prehabilitation group compared with the rehabilitation group (53 *vs*. 15%, adjusted *P= 0.006*). Complication rates and duration of hospital stay were similar. The difference between baseline and 8-week 6-min walking test was significantly higher in the prehabilitation compared with the rehabilitation group (+23.7 m [SD, 54.8] *vs*. -21.8m [SD, 80.7]; mean difference 45.4 m [95% CI, 13.9 to 77.0]). A higher proportion of the prehabilitation group were also recovered to or above baseline exercise capacity at 8 weeks compared with the rehabilitation group (84 *vs*. 62%, adjusted *P=0.049*). **Conclusions:** Meaningful changes in postoperative functional exercise capacity can be achieved with a prehabilitation program

ANTIOXIDANTS (BASEL) 2014 DEC 12;3(4) :843-65

13-A CYSTEINE-RICH WHEY SUPPLEMENT (IMMUNOCAL®) DELAYS DISEASE ONSET AND PREVENTS SPINAL CORD GLUTATHIONE DEPLETION IN THE hSOD1 (G93A) MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Abstract: Depletion of the endogenous antioxidant, glutathione (GSH), underlies progression of the devastating neurodegenerative disease, amyotrophic lateral sclerosis (ALS). Thus, strategies aimed at elevating GSH may yield new therapeutics for ALS. Here, we investigated the effects of a unique non-denatured whey protein supplement, Immunocal®, in the transgenic Gly position 93 to Ala (G93A) mutant hSOD1 (hSOD1(G93A)) mouse model of ALS. Immunocal® is rich in the GSH precursor, cystine, and is therefore capable of bolstering GSH content. Transgenic hSOD1 (G93A) mice receiving Immunocal® displayed a significant delay in disease onset compared to untreated hSOD1(G93A) controls. Additionally, Immunocal® treatment significantly decreased the rate of decline in grip strength and prevented disease-association reduction in whole blood and spinal cord tissue GSH levels in end-stage hSOD1(G93A) mice. However, Immunocal® did not extend survival, likely due to its inability to preserve the mitochondrial GSH pool in spinal cord. Combination treatment with Immunocal and the anti-glutamatergic compound, riluzole, delayed disease onset and extended survival in hSOD1(G93A) mice. These findings demonstrate that sustaining tissue GSH with Immunocal® only modestly delays disease onset and slows the loss of skeletal muscle strength in hSOD1(G93A) mice. Moreover, the inability to Immunocal® to rescue mitochondrial GSH in spinal cord provides a possible mechanism for its lack of effect on survival and is a limiting factor in the potential utility of this supplement as a therapeutic for ALS.

KEYWORDS:
Immunocal®; amyotrophic lateral sclerosis; cysteine; glutathione; oxidative stress; whey protein

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14-THE CYSTEINE-RICH WHEY PROTEIN, IMMUNOCAL®, RESCUES REELIN EXPRESSION IN THE hAPPSweInd TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE.

Poster

J CLIN AESTHET DETERMATOL, 2013;6(10):23-26).

15-PSORIASIS IMPROVEMENT IN PATIENTS USING GLUTATHIONE-ENHANCING, NONDENATURED WHEY PROTEIN ISOLATE
A Pilot Study

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ABSTRACT

Background: Psoriasis is a common autoimmune disease with enhanced systemic inflammation and heightened levels of oxidative stress. Glutathione is the major antioxidant in human cells. **Objectives:** To determine if a nondenatured bioactive whey protein isolate previously demonstrated to increase glutathione levels can clinically improve patients with psoriasis vulgaris. **Methods:** A single site prospective, non-blinded trial. Seven patients with psoriasis were recruited to take a nondenatured bioactive whey protein isolate, 20g orally per day, in addition to their current treatments, if any. Psoriasis Area and Severity Index scores and photographs were taken at baseline and monthly for three months. **Results:** Patients with psoriasis were found to have a beneficial clinical improvement, whether they were on existing topical therapy, narrowband ultraviolet B, or no other treatment. **Conclusion:** The positive preliminary outcomes from this pilot study suggest a randomized, double-blind, clinical trial would be worthwhile in evaluating whether this protein isolate would result in statistically significant improvement for patients with psoriasis.

RECENT PAT CNS DRUG DISCOV. 2012 DEC;7(3):230-5

16-IMMUNOCAL® AND PRESERVATION OF GLUTATHIONE AS A NOVEL NEUROPROTECTIVE STRATEGY FOR DEGENERATIVE DISORDERS OF THE NERVOUS SYSTEM

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ABSTRACT

Background: Oxidative stress and glutathione (GSH) depletion are both recognized as significant contributors to the pathogenesis of many devastating neurodegenerative diseases. In particular, mitochondrial dysfunction leads to the aberrant production and accumulation of reactive oxygen species (ROS) which are capable of oxidizing key cellular proteins, lipids, and DNA, ultimately triggering cell death. In addition to other roles that it plays in the cell, GSH functions as a critical scavenger of these ROS. Therefore, GSH depletion exacerbates cell damage due to free radical generation. Strategies that increase or preserve the levels intracellular GSH have been shown to act in a neuroprotective manner, suggesting that augmentation of the available GSH pool may be a promising therapeutic target for neurodegeneration. This review discusses the capacity of a cystine-rich, whey protein supplement (Immunocal®) to enhance the de novo synthesis of GSH in neurons, and

highlights its potential as a novel therapeutic approach to mitigate the oxidative damage that underlies the pathogenesis of various neurodegenerative diseases. Additionally, this review discusses various patents from 1993 to **2012** both with Immunocal® and other methods that modulate GSH in neurodegeneration.

RECENT PAT CNS DRUG DISCOV. 2012 DEC: 7(1)

Editorial
17-THERAPEUTIC ANTIOXIDANTS FOR NEURODEGENERATIVE DISEASE

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The aberrant production of reactive oxygen species (ROS) within a cell can cause significant oxidative damage to key cellular proteins, lipids, and DNA. Harmful free radical species like ROS can be generated intrinsically via inadvertent "leakage" from the mitochondrial electron transport chain or by oxidant-generating enzymatic systems like NADPH oxidase, xanthine oxidase, glucose oxidase, or nitric oxide synthase. Alternatively, noxious free radicals can be generated by extrinsic sources such as toxins or reactive inflammatory cells. Regardless of their source, ROS and other free radical species must be scavenged by intracellular antioxidant systems to protect the cell from oxidative damage and consequent cell death. To this end, the cell has developed a large repertoire of antioxidant defense mechanisms that are normally able to keep ROS in check; however, during many disease states these antioxidant defenses are often overwhelmed and the cell succumbs to oxidative stress. This certainly appears to be the case in many types of neurodegenerative disease including Alzheimer's disease, Parkinson's disease, Huntington's disease, and amyotrophic lateral sclerosis, to name a few. For each of these disorders, oxidative stress is a significant factor in the neuronal death underlying disease pathogenesis. In addition, oxidative stress is hypothesized to play a substantial role in aging which is a major risk factor for neurodegeneration. Thus, it is not surprising that strategies either to bolster endogenous antioxidant defenses or to provide exogenous free radical scavengers are currently under intense investigation as potential therapies for neurodegeneration.

J AGRIC FOOD CHEM 58:12729-12734 (2010)

18-EFFECTS OF WHEY PROTEIN CONCENTRATE (WPC) ON THE DISTRIBUTIONS OF LYMPHOCYTE SUBPOPULATIONS IN RATS WITH EXCESSIVE ALCOHOL INTAKE.

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Abstract: To investigate the effects of whey protein concentrate (WPC) on antioxidant statuses and the lymphocyte subpopulations in the rats with alcohol intake, the antioxidant statuses in the peripheral blood (PB) and the lymphocyte subpopulations in the PB, spleen,

and bone marrow (BM) of the rats fed with WPC (0.334 g/kg) and alcohol (6 g/kg) for 3 months were analyzed. Results showed that the effects of WPC on the glutathione peroxidase and glutathione in the PB, the T and B cells in the spleen, and the B cells in the BM were more apparent in the rats with alcohol intake; however, they are not apparent in the controls. Taken together, our results indicated that the immunity of rats might be enhanced by the increased antioxidant ability after WPC supplementation and the effects of WPC on the lymphocyte subpopulations were mainly in the spleen and BM and not in the PB.

JOURNAL OF GASTROENTEROLOGY & HEPATOLOGY 24:1045-1050 (2009)

19-OPEN-LABELED PILOT STUDY OF CYSTEINE-RICH WHEY PROTEIN ISOLATE SUPPLEMENTATION FOR NONALCOHOLIC STEATOHEPATITIS PATIENTS

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Background and Aims: Glutathione (GSH) depletion contributes to liver injury and development of steatohepatitis. Undenatured cysteine-rich whey protein isolate has been clinically proven to raise GSH in several patient groups. The aim of this study was to evaluate the effect of oral supplementation with whey protein on patients with nonalcoholic steatohepatitis (NASH).

Methods: In an open-labeled clinical trial, 38 patients (18 male, 20 female; mean age 48 ± 14 years) with NASH confirmed by computed tomography measurements and liver biochemistries were given with a daily dose of 20g whey protein isolate for 12 weeks.

Results: A significant reduction in alanine aminotransferase (ALT) (64 ± 72 *vs* 46 ± 36, *P*=0.016) and aspartate aminotransferase (AST) (45 ± 49 *vs* 33 ± 18, *P*=0.047) were observed. Plasma glutathione and total antioxidant capacity increased significantly at the end of study (53 ± 11 *vs* 68 ± 11, P< 0.05 and 1.26 ± 0.10 *vs* 2.03 ± 0.10, *P*< 0.05). Liver attenuation index improved from -13.4 ± 11.1 to -9.7 ± 13.1 (*P* = 0.048). Hepatic macrovesicular steatosis decreased significantly after 12 weeks of supplementation (33.82 ± 12.82 *vs* 30.66 ± 15.96, *P*=0.046). Whey protein isolate was well tolerated. No serious adverse events were observed.

Conclusions: The results indicate that oral supplementation of cysteine-rich whey protein isolate leads to improvements in liver biochemistries, increased plasma GSH, total antioxidant capacity and reduced hepatic macrovesicular steatosis in NASH patients. The results support the role of oxidative stress in the pathogenesis of this disease.

PEDIATR BLOOD CANCER 2008;50:490-493

20-BRINGING EVIDENCE TO COMPLEMENTARY AND ALTERNATIVE MEDICINE IN CHILDREN WITH CANCER: FOCUS ON NUTRITION-RELATED THERAPIES

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Children with cancer frequently use complementary and alternative medicine (CAM), especially in conjunction with conventional therapy. Dietary supplements are a commonly used CAM modality, with the prevalence of supplement use ranging from 35% to 50% of children with cancer in surveys completed in the United States. Less is known about the use of dietary supplements in developing countries. The evidence for some dietary supplements providing some benefit to children with cancer is reviewed. Preliminary studies have shown that antioxidant status may affect chemotherapy tolerance in children with acute lymphoblastic leukemia. Other supplements, including TRAUMEEL S®, glutamine, vitamin E, Immunocal®, colostrum, and probiotics, may help to reduce gastrointestinal toxicities of chemotherapy and radiation. However, more definitive evidence is needed. Most dietary supplements have not been tested adequately to determine their safety and efficacy, with even less understood about their potential interactions with conventional chemotherapy and radiation. With the greater use of dietary supplements by patients with cancer, increasing scientific attention is being paid to the investigation of these therapies. But research on dietary supplements is complex and usually more difficult than that on conventional medications. Strong research designs are
critical in obtaining information that will ultimately influence clinical practice and public awareness.

ANTIOXIDANTS & REDOX SIGNALING, 10:661-675 (2008)

21- ABERRANT INSULIN RECEPTOR SIGNALING AND AMINO ACID HOMEOSTASIS AS A MAJOR CAUSE OF OXIDATIVE STRESS IN AGING

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ABSTRACT – The mechanisms leading to the increase in free-radical-derived oxidative stress in "normal aging" remained obscure. Here we present our perspective on studies from different fields which reveal a previously unnoticed vicious cycle of oxidative stress. The plasma cysteine concentrations during starvation in the night and early morning hours (the *postabsorptive state*) decreases with age. This decrease is associated with a decrease in tissue concentrations of the cysteine derivative and quantitatively important antioxidant glutathione. The decrease in cysteine reflects changes in the autophagic protein catabolism which normally ensures free amino acid homeostasis during starvation. Autophagy is negatively regulated by the insulin receptor signaling cascade, which is enhanced by oxidative stress in the absence of insulin. This synopsis of seemingly unrelated processes reveals a novel mechanism of progressive oxidative stress in which decreasing antioxidant concentrations and increasing basal (*postabsorptive*) insulin receptor signaling activity compromise not only the autophagic protein catabolism but also the activity of FOXO transcription factors, i.e. two functions which

were found to have an impact on lifespan in several animal models of aging. In addition, the aging-related decrease in glutathione level is likely to facilitate certain "secondary" disease-related mechanisms of oxidative stress. Studies on cysteine supplementation show therapeutic promise.

ANTIOXIDANTS & REDOX SIGNALING, 10:395-402, (2008)

22-CYSTEINE-RICH PROTEIN REVERSES WEIGHT LOSS IN LUND CANCER PATIENTS RECEIVING CHEMOTHERAPY OR RADIOTHERAPY

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ABSTRACT – Oxidative stress plays a role in the tumor-cytotoxic effect of cancer chemotherapy and radiotherapy and also in certain adverse events. In view of these conflicting aspects, a double-blind trial over 6 months has been performed to determine whether a cysteine-rich protein (IMN1207) may have a positive or negative effect on the clinical outcome if compared with casein, a widely used protein supplement low in cysteine. Sixty-six patients with Stage IIIB-IV non-small cell lung cancer were randomly assigned to IMN1207 or casein. Included were patients with a previous involuntary weight loss of ≥3%, Karnofsky status ≥70, and an estimated survival of > 3 months. Thirty-five lung cancer patients remained on study at six weeks. Overall compliance was not different between treatment arms (42-44% or 13g/day). The patients treated with the cysteine-rich protein had a mean increase of 2.5% body weight while casein-treated patients lost 2.6% (*P=0.049*). Differences in secondary end points included an increase in survival, hand grip force and quality of life. Adverse events were mild or moderate. Further studies will have to show whether the positive clinical effects can be confirmed and related to specific parameters of oxidative stress in the host.

PEDIATR BLOOD CANCER, 50:447-450 (2008)

23-CHILDREN'S ONCOLOGY GROUP (COG) NUTRITION COMMITTEE

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Children's Oncology Group (COG) Nutrition Committee was established to further the knowledge of nutrition in children with cancer by education and conduct of clinical trials. A survey of COG institutions revealed lack of conformity in evaluation and categorization of

nutritional status, and criteria for nutritional intervention. The Committee subsequently established specific categories of malnutrition (Underweight and Overweight) based on ideal body weight or body mass index. An algorithm was developed as a guideline for nutritional intervention as well as references and resources for determining estimated needs. The Committee embarked on concepts for clinical trials of nutritional interventions. The first pilot study, evaluating the feasibility of using an immunoneutraceutical precursor for glutathione production, has been completed. The study showed weight gain and improvement in glutathione status. A pilot trial of proactive enteral feeding for patients at high risk of malnutrition has commenced. The Committee believes that nutrition is relevant to all aspects of cancer control. The paucity of nutritional investigation in children with cancer needs to be rectified. **Key words**: cancer, children; nutrition.

JANA VOL. 11, NO. 1, (2008)

24-0RAL TOLERABILITY OF CYSTEINE-RICH WHEY PROTEIN ISOLATE IN AUTISM – A PILOT STUDY

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ABSTRACT – **Purpose**: To examine the tolerability of non-denatured whey protein isolate (NWPI) in children with autism. Many children with autism are low in glutathione and have higher levels of oxidative stress. NWPI can raise glutathione levels and reduce oxidative stress. However, anecdotal reports suggest that NWPI may be problematic in children with autism because it contains cysteine and other sulfurated amino acids.

Methods: A 6-week open-label trial was conducted, supplementing 10 children with autism or autism spectrum disorder (ASD), 3-15 years of age, with NWPI (Immunocal®). To measure possible side effects, procedures that examined the frequency, intensity, and types of side effects, as well as behavioral measures, were completed at baseline, and at days 3, 14, 30 and 45.

Results: Seven of the ten children took the supplement over the six-week trial and tolerated it well. Two children discontinued after two weeks due to possible side effects: one due to gastrointestinal disturbance and one due to being less responsive to parents. Another child discontinued due to difficulty of administering the product.

Conclusion: This study suggests that NWPI can be used as a supplement for this small population of children with autism without high rates of side effects, which means that further studies to determine its safety and efficacy in larger populations might yield the same promising result. Larger studies are planned to determine its efficacy in raising glutathione levels.

J AGRIC FOOD CHEM. 56:8141-8147 (2008)

25-EFFECTS OF ALCOHOL-INDUCED HUMAN PERIPHERAL BLOOD MONONUCLEAR CELL (PBMC) PRETREATED WHEY PROTEIN CONCENTRATE (WPC) ON OXIDATIVE DAMAGE.

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Abstract: Excessive alcohol consumption can induce apoptosis in a variety of tissues and influence the antioxidant status in peripheral blood mononuclear cells (PBMC). This paper investigates the effects of whey protein concentrate (WPC) pretreated in PBMC on the apoptosis and antioxidant status after the treatment of alcohol. The results show that the percentages of apoptotic cells in the alcohol-treated group were higher than those in the group without alcohol treatment. Additionally, there was higher glutathione (GSH) peroxidase (GPx) activity when the PBMC were treated with 300 mg/dL of alcohol. With regards to the activity of GSH reductase (GRx), there was higher activity in the group pretreated with WPC than in the group with the treatment of alcohol only. On the contrary, the levels of GSH were reduced after the treatment of alcohol, but there was a higher level of GSH in the group pretreated with WPC. In this study, it was found that the increased level of GSH in PBMC might not be attributed to the effect of GRx because there was still a higher level of GSH in the group with the treatment of WPC and BCNU (a GRx inhibitor) in this study. The results indicated that PBMC pretreated with WPC might ameliorate alcohol-induced effects such as imbalance of the antioxidant status.

FOOD CHEM TOXICOL. 44:574-578, (2006)

26-WHEY PROTEIN CONCENTRATE PROMOTES THE PRODUCTION OF GLUTATHIONE (GSH) BY GSH REDUCTASE IN THE PC12 CELL LINE AFTER ACUTE ETHANOL EXPOSURE.

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Abstract: Excessive ethanol consumption may increase the production of reactive oxygen species (ROS), which results in the damage of tissues, especially the neurons and glial cells in the central nervous system (CNS). The purpose of this study is to evaluate the effects of whey protein concentrate (WPC) on the glutathione (GSH) status after acute ethanol exposure in the pheochromocytoma (PC12) cell line. In this study, we assayed the cell viability, the percentage of lactate dehydrogenase released (% LDH released), the level of GSH, and the activity of GSH reductase (GRx). The results showed that with the supplement of WPC, the cell viability displayed no significant difference after acute exposure of ethanol in groups with or without ethanol treatment. The ethanol-induced cytotoxicity showed a slight decrease, and the level of GSH showed a significant increase. The activity of GRx significantly increased when 0.1, 10mg/ml of WPC was supplied. In conclusion, these results suggest that WPC in a moderate concentration should be a precursor agent to promote the production of GSH and will enhance the antioxidant capacity in the PC12 cell line.

PHIL. TRANS. R. SOC. B. 360, 2355-2372 (2005).

27-OXIDATIVE STRESS AND AGEING: IS AGEING A CYSTEINE DEFICIENCY SYNDROME?

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ABSTRACT – Reactive oxygen species (ROS) are constantly produced in biological tissues and play a role in various signaling pathways. Abnormally high ROS concentrations cause oxidative stress associated with tissue damage and dysregulation of physiological signals. There is growing evidence that oxidative stress increases with age. It has also been shown that the life span of worms, flies and mice can be significantly increased by mutations, which impede the insulin receptor signaling cascade. Molecular studies revealed that the insulin-independent basal activity of the insulin receptor is increased by ROS and downregulated by certain antioxidants. Complementary clinical studies confirmed that supplementation of the glutathione precursor cysteine decreases insulin responsiveness in the fasted state. In several clinical trials, cysteine supplementation improved skeletal muscle functions, decreased the body fat/lean body mass ratio, decreased plasma levels of the inflammatory cytokine tumour necrosis factor α (TNF-α), improved immune functions, and increased plasma albumin levels. As all these parameters degenerated with age, these findings suggest: (i) that loss of youth, health and quality of life may be partly explained by a deficit in cysteine and (ii) that the dietary consumption of cysteine is generally suboptimal and *everybody* is likely to have a cysteine deficiency sooner or later.

MED. SCI. SPORTS EXERC., VOL. 37, NO. 9, PP. 1468-1473, 2005.

28-EFFECTS OF CYSTEINE DONOR SUPPLEMENTATION ON EXERCISE-INDUCED BRONCHOCONSTRICTION

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ABSTRACT – ***Purpose***: Reactive oxygen/nitrogen species (ROS/RNS) in resident airway cells may be important in bronchoconstriction following exercise. Glutathione (GSH) is a major lung antioxidant and could influence pathological outcomes in individuals with exercise-induced bronchoconstriction (EIB). This study examined the effects of supplementation with undenatured whey protein (UWP) in subjects exhibiting airway narrowing following eucapnic voluntary hyperventilation (EVH), a surrogate challenge for diagnosis of EIB. UWP is a cysteine donor that augments GSH production. ***Methods***: In a randomized, double-blind, placebo-controlled study, 18 EIB-positive subjects (age: 25.2 ± 9.01 yr; weight: 77.3 ± 18.92 kg; height: 1.7 ± 0.09 m) with post-EVH falls of ≥ 10% in FEV received 30 g UWP (TX) or casein placebo (PL)/d. Subjects performed 6-min EVH challenges before and after 4 and 8 wk of supplementation. Exhaled nitric oxide (eNO) was measured serially before spirometry and at 1-wk intervals. Spirometry was performed pre- and 5, 10, and 15 min postchallenge. ***Results***: Subjects exhibited significant mean improvement in postchallenge falls in FEB from 0 wk (-2.6 ± 12.22%) with TX at 4 (-18.9 ± 12.89%, $P<0.05$) and 8 wk (-16.98 ± 11.61%, $P<0.05$) and significant mean reduction in post-EVH peak falls in FEF from 0 wk (-40.6 ± 15.28%) with TX at 4 (-33.1 ± 17.11%, $P<0.01$)

and 8 (-29.7 ± 17.42%, $P<0.05$) wk. No changes in FEV or FEF were observed in the PL group at any time point. Mean eNO for PL and TX groups at 0, 4, and 8 wk (46.8 ± 31.33, 46.5 ± 35.73, 49.3 ± 37.12 vs 35.2 ± 26.87, 29.1 ± 17.26, 34.7 ± 21.11 ppb, respectively) was not significantly different. ***Conclusions***: UWP may augment pulmonary antioxidant capacity and be therapeutically beneficial in individuals exhibiting EIB, as postchallenge pulmonary function improved with supplementation. The lack of significant change in eNO suggests that the pulmonary function improvements from UWP supplementation are independent of eNO.

Author keywords: Asthma, inflammation, pulmonary function, whey protein, glutathione.

ANTICANCER RESEARCH 24: 553-554 (2004)

29-MOLECULAR PATHOGENESIS AND PREVENTION OF PROSTATE CANCER

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ABSTRACT – Studies in laboratory animals indicate inhibition of chemically-induced carcinoma by cystine-rich diets enhancing the cysteine-GSH antioxidant system. The progression of carcinoma of the prostate is also inhibited by these diets, which were later found to raise the level of GSH in the prostate epithelium of man. New data presented at the July 13, 2003 meeting of the American Association for Cancer Research indicates that higher levels of total cysteine in plasma may predict a reduced risk for breast cancer. This prospective investigation was conducted among 32,000 women in the Nurses Health study. The previously reported prostate cancer data appears then not to be strictly gender-related as the antioxidant role of the cysteine – GSH system may also apply to breast cancer prevention.

JOURNAL OF CYSTIC FIBROSIS, VOL 2, ISSUE 4, DECEMBER 2003

30-IMPROVED GLUTATHIONE STATUS IN YOUNG ADULT PATIENTS WITH CYSTIC FIBROSIS SUPPLEMENTED WITH WHEY PROTEIN

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ABSTRACT – *Background*: The lung disease of cystic fibrosis is associated with a chronic inflammatory reaction and an over abundance of oxidants relative to antioxidants. Glutathione functions as a major frontline defense against the build-up of oxidants in the lung. This increased demand for glutathione (GSH) in cystic fibrosis may be limiting if nutritional status is compromised. We sought to increase glutathione levels in stable patients with cystic fibrosis by supplementation with a whey-based protein. *Methods*: Twenty-one patients who were in stable condition were randomly assigned to take a whey protein isolate (Immunocal, 10 g twice a day) or casein placebo for 3 months. Peripheral lymphocyte GSH was used as a marker of lung

GSH. Values were compared with nutritional status and lung parameters. *Results*: At baseline there were no significant differences in age, height, weight, percent ideal body weight or percent body fat. Lymphocyte GSH was similar in the two groups. After supplementation, we observed a 46.6% increase from baseline ($P<0.05$) in the lymphocyte GSH levels in the supplemented group. No other changes were observed. *Conclusion*: The results show that dietary supplementation with a whey-based product can increase glutathione levels in cystic fibrosis. This nutritional approach may be useful in maintaining optimal levels of GSH and counteract the deleterious effects of oxidative stress in the lung in cystic fibrosis.

Author keywords: Glutathione, Cystic fibrosis; Whey

ANTICANCER RESEARCH *23*: 1411-1416 (2003).

31-THE ANTIOXIDANT SYSTEM

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ABSTRACT – The glutathione (GSH) antioxidant system is the principal protective mechanism of the cell and is a crucial factor in the development of the immune response by the immune cells. Experimental data demonstrate that a cysteine-rich whey protein concentrate represents an effective cysteine delivery system for GSH replenishment during the immune response. Animal experiments showed that the concentrates of whey protein also exhibit anticancer activity. They do this via the GSH pathway, the induction of p53 protein in transformed cells and inhibition of neoangiogenesis.

CAN J CARDIOL VOL 19, NO 10, 1163-1168, SEPTEMBER 2003.

32-MILK WHEY PROTEIN DECREASES OXYGEN FREE RADICAL PRODUCTION IN A MURINE MODEL OF CHRONIC IRON-OVERLOAD CARDIOMYOPATHY

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ABSTRACT – Chronic iron overload is a major cause of organ failure worldwide, but its pathogenesis remains to be elucidated.

To examine in an experimental murine model of iron-overload cardiomyopathy the relation between milk whey protein and, first, the production of reactive oxygen free radical species and, second, antioxidant reserve status.

B6D2F1 mice were randomly assigned to four treatment groups (n=8 per treatment group): placebo control; iron only; whey only; and iron with whey. Reactive oxygen free radical species in the heart were quantified by the cytotoxic aldehydes malondialdehyde (MDA), 4-hydroxy-nonenal (HNE) and hexanal, while antioxidant reserve status was quantified by glutathione (GSH) and glutathione peroxidase (GPx) activity in the heart tissue.

Significantly decreased concentrations (pmol/100 mg wet weight tissue) of MDA (2468 ± 261), HNE (912 ± 38) and hexanal (5385 ± 927) were observed in the heart tissue of the group receiving iron with whey, in comparison with the iron-only treatment group (MDA 9307 ± 387, HNE 1416 ± 157, hexanal 14,874 ± 2955; P<0.001). Significantly increased GPx (141 ±

38 IU/L) and GSH (521 ± 136 IU/L) activity were observed in mice receiving iron with whey, in comparison with mice receiving iron only (GPx 100 ± 10 IU/L, GSH 446 ± 33 IU/L; P<0.001).

Mice receiving iron treatments with whey supplementation had significantly lower concentrations of cytotoxic aldehydes and significantly higher cardiac levels of GPx and GSH activity than did iron-only treated mice. Additional basic research is warranted to examine the exact mechanisms by which milk whey protein protects the heart.

ANTICANCER RESEARCH 20: 4785-4792, 2000.

33-WHEY PROTEIN CONCENTRATE (WPC) AND GLUTATHIONE MODULATION IN CANCER TREATMENT

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ABSTRACT - The glutathione (GSH) antioxidant system is foremost among the cellular protective mechanisms. Depletion of this small molecule is a common consequence of increased formation of reactive oxygen species during increased cellular activities. This phenomenon can occur in the lymphocytes during the development of the immune response and in the muscular cells during strenuous exercise. It is not surprising that so much research has been done, and is still being done on this small tripeptide molecule. Whey protein concentrate has been shown to represent an effective and safe cysteine donor for GSH replenishment during GSH depletion in immune deficiency states. Cysteine is the crucial limiting amino acid for intracellular GSH synthesis. Animal experiments showed that the concentrates of whey proteins also exhibit anti-carcinogenesis and anticancer activity. They do this via their effect on increasing GSH concentration in relevant tissues, and may have anti-tumor effect on low volume of tumor via stimulation of immunity through the GSH pathway. It is considered that oxygen radical generation is frequently a critical step in carcinogenesis, hence the effect of GSH on free radicals as well as carcinogen detoxification, could be important in inhibiting carcinogenesis induced by a number of different mechanisms. Case reports are presented which strongly suggest an anti-tumor effect of a whey protein dietary supplement in some urogenital cancers. This non toxic dietary intervention, which is not based on the principles of current cancer chemotherapy, will hopefully attract the attention of laboratory and clinical oncologists.

NUTRITION AND CANCER, 2000, VOL 38(2):200-208.

34-ENHANCING EFFECT OF PATENTED WHEY PROTEIN ISOLATE (IMMUNOCAL®) ON THE CYTOTOXICITY OF ANTI-CANCER DRUG

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ABSTRACT – To determine the enhancing effect of a whey protein isolate on the cytotoxicity of a potential anti-cancer drug. baicalein, human hepatoma cell line HepG2 was assigned to grow in different media for four days, followed by the investigation of cell

growth and apoptosis. Excluding the control group with normal medium, other three treatment media included whey protein isolate (marketed as Immunocal™) medium, baicalein medium, and combined medium containing both Irnmunocal™ and baicalein. MTT assay indicated that cells grew in combined medium had a significantly lower survival rate compared to the cells grew in baicalein medium; in contrast, for the cells grew in Immunocal™ group, there was no significant difference on survival rate. In the investigation of apoptosis. compared to the cells in baicalein medium, cells in combined medium showed a higher phosphatidylserine exposure, lower rnitochondrial transmembrane potential and nearly 13 times more cells were detected undergoing apoptosis. We also demonstrated that Immunocal™ was able to reduce glutathione in HepG2 by 20% to 40% and regulated the elevation of glutathione, which was in response to baicalein. In conclusion, Immunocal™ seemed to enhance the cytotoxicity of baicalein by inducing more apoptosis, this increase in apoptotic cells may be in association with the depletion of GSH in HepG2. This is the first study to demonstrate, in vitro, that Immunocal™ may function as an adjuvant in cancer treatments.

CHEST 2000, MAR; 117(3):914-6.

35-TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE WITH A CYSTEINE DONOR PROTEIN SUPPLEMENT: A CASE REPORT

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ABSTRACT:Oxidant/antioxidant imbalance can occur in obstructive airways disease, as a result of ongoing inflammation. Glutathione plays a major role in pulmonary antioxidant protection. As an alternative or complement to anti-inflammatory therapy, augmenting antioxidant protection could diminish the effects of inflammation. We describe a case of a patient with obstructive lung disease, responsive to corticosteroids, with low whole blood glutathione levels. Following one month of supplementation with a whey-based oral supplement, designed to provide glutathione precursors, whole blood glutathione levels and pulmonary function significantly and dramatically increased. The potential for such supplementation in pulmonary inflammatory conditions deserves further study.

JOURNAL OF APPLIED PHYSIOLOGY, *87*: 1381-1385, 1999

36-THE EFFECT OF SUPPLEMENTATION WITH A CYSTEINE DONOR ON MUSCULAR PERFORMANCE

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ABSTRACT: Oxidative stress contributes to muscular fatigue. Glutathione (GSH) is the major intracellular antioxidant, whose biosynthesis is dependent upon 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 m) were studied pre- and 3 months post-supplementation with either Immunocal (20 gm/day) or casein placebo. Muscular performance was assessed by whole leg isokinetic cycle testing, measuring Peak Power and 30-sec 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-sec Work Capacity). Follow-up data on 18 subjects (9 Immunocal, 9 placebo) were analyzed. Both Peak Power (mean±se: 13±3.5%, $p<0.02$) and 30-sec Work Capacity (13±3.7%, $p<0.03$) increased significantly in the Immunocal group, with no change (2±9.0 and 1±9.3%) in the placebo group. Lymphocyte GSH also increased significantly in the Immunocal group (35.5±11.04%, $p<0.02$) with no change in the placebo group (-0.9±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.

Key words: oxidative stress, exercise

MEDICAL HYPOTHESES (1999) *53(4):* 347-349.

37-COMPETITION FOR GLUTATHIONE PRECURSORS BETWEEN THE IMMUNE SYSTEM AND THE SKELETAL MUSCLE: PATHOGENESIS OF CHRONIC FATIGUE SYNDROME

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SUMMARY - 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.

OXIDATIVE STRESS IN CANCER, AIDS, AND NEURODEGENERATIVE DISEASES – LUC MONTAGNIER ET AL., (ED.) MARCEL DEKKER INC., NEW YORK: 447-461, 1998

38-NUTRICEUTICAL MODULATION OF GLUTATHIONE WITH A HUMANIZED NATIVE MILK SERUM PROTEIN ISOLATE, IMMUNOCAL™: APPLICATION IN AIDS AND CANCER

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ABSTRACT – The biological activity of the proteins isolated from cow's milk in Immunocal™ depends on the preservation of those labile proteins which share with the predominant human milk proteins the same extremely rare glutathione (GSH)-promoting components. Cellular GSH depletion has been implicated in the pathogenesis of a number of degenerative conditions and disease states including Parkinson's, Alzheimer's, arteriosclerosis, cataracts, cystic fibrosis, malnutrition, aging, AIDS, and cancer.

This newly discovered nutriceutical modulation of GSH by the use of humanized native milk serum protein isolate of bovine origin in AIDS and cancer may well find other applications in disease where oxidative stress and pathology of GSH metabolism are largely implicated. In a pilot study, this type of whey protein concentrate was found to be well tolerated in children with AIDS and wasting syndrome and was found associated with an improvement of the nutritional status of the patient. Moreover, the GSH promoting activity on the peripheral blood lymphocyte of this protein concentrate was validated in patients with initial low GSH levels. Extensive pharmaco-epidemiological study of GSH metabolism and standardized methods of measurement of intracellular GSH applicable in clinical trials are needed in order to better define the clinical application of this new type of therapy.

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

39-TREATMENT OF CHRONIC HEPATITIS USING WHEY PROTEIN (NON-HEATED)

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In an open study, the clinical efficacy of whey protein (Immunocal: cysteine content; 7.6-fold that of casein) isolated from fresh milk and purified without being heated was evaluated based on liver function test, immunological parameters, plasma or lymphocyte GSH concentrations and hepatitis virus markers 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 given for 2 weeks prior to the start of -supplement with Immunocal food (induction period) and *for* 4 weeks after the end (follow-up period). The effects of Immunocal food on various clinical parameters

were examined at 4-week intervals for 18 weeks to evaluate the efficacy of Immunocal. As a result, serum ALT activity decreased in 6 of 8 patients with chronic hepatitis B 12 weeks after the start of supplement with Immunocal food. Plasma GSH concentrations were increased in 5 of the 8 patients. Serum . concentrations of lipid peroxides significantly decreased 8 weeks after Immunocal food. Serum IL-2 levels began to increase 8 weeks and remained high even after supplement with Immunocal -food had ended. Furthermore, NK activity was significantly increased. However, an item correlating with reduced serum ALT activity could not be clarified. In 17 patients with chronic hepatitis C, there wore no significant Immunocal-related changes in liver function test or immunological parameters. These findings suggest that long-term supplement with Immunocal alone may be effective for patients with chronic hepatitis B, and a further clinical study that long-term combination therapy with Immunocal and other agents including interferon may be effective for those with chronic hepatitis C should be performed.

ANTICANCER RESEARCH 16:1095-1100 (1996).

40-*IN VITRO* SELECTIVE MODULATION OF CELLULAR GLUTATHIONE BY A HUMANIZED NATIVE MILK PROTEIN ISOLATE IN NORMAL CELLS AND RAT MAMMARY CARCINOMA MODEL

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Abstract: We report the *in vitro* selective inhibitory activity of a humanized whey protein concentrate IMMUNOCAL™ on growth of mammary carcinoma cells and Jurkat T cells in comparison to normal peripheral blood mononuclear cells. We related this inhibitory activity to a selective depletion of intracellular glutathione synthesis. The use of humanized whey protein concentrate as a food supplementation may have direct implication in clinical trials with adjuvant chemotherapy.

Glutathione (GSH) accounts for more than 90% of total intracellular non-protein sulfhydryl and is critical in a variety of cellular defense functions including protection from toxic oxygen species and detoxification of various xenobiotics. Tumor cell GSH concentration may be among the determinant of the cytotoxicity of many chemotherapeutic agents and of radiation, and an increase in GSH concentration appears to be at least one of the mechanisms of acquired drug resistance to chemotherapy.

Therapeutic elevation of normal cell GSH levels has also been investigated as a means to reduce the toxicity associated with a wide variety of compounds of both endogenous and exogenous origin.

GSH may be increased by different methods including delivery of L-Cystine, a rare limiting amino acid in GSH synthesis. This is difficult since cysteine is toxic, it is not transported efficiently into cells, and is oxidized spontaneously at neutral pH.

Attempts to cancer treatment based on modulation of GSH concentration in tumor cells must take into consideration the glutathione status and the rate of GSH synthesis in these cells. It is well known that rapid GSH synthesis in tumor cells is associated with high rates of cellular proliferation. Depletion of tumor GSH *in vivo* decreases the rate of cellular proliferation and inhibits cancer growth. In practice it is difficult to reduce GSH sufficiently in a tumor *in vivo* without placing the normal tissue at risk.

Numerous studies have demonstrated that GSH can be differently manipulated in normal versus tumor cell line. Dependent upon the method of GSH manipulation protection could be

demonstrated in normal but not in tumor cell line.

In this report we demonstrate that it is possible to selectively modulate *in vivo* GSH synthesis in normal cells compared to cancer cells with a humanized Whey Protein Concentrate (HWPC) and that this selective GSH modulation has an impact on cells proliferation.

Anticancer RESEARCH *15*: 2643-2650, 1995.

41-THE USE OF A WHEY PROTEIN CONCENTRATE IN THE TREATMENT OF PATIENTS WITH METASTATIC CARCINOMA: A PHASE I-II CLINICAL STUDY

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ABSTRACT. Glutathione (GSH) concentration is high in most tumor cells and this may be an important factor in resistance to chemotherapy. Previous in-vitro and animal experiments have shown a differential response of tumor versus normal cells to various cysteine delivery systems. More specifically, an in-vitro assay showed that at concentrations that induce GSH synthesis in normal human cells, a specially prepared whey protein concentrate, Immunocal™, caused GSH depletion and inhibition of proliferation in human breast cancer cells. On the basis of this information five patients with metastatic carcinoma of the breast, one of the pancreas and one of the liver were fed 30 grams of this whey protein concentrate daily for six months. In six patients the blood lymphocyte GSH levels were substantially above normal at the outset, reflecting high tumor GSH levels. Two patients (#1, #3) exhibited signs of tumor regression, normalization of haemoglobin and peripheral lymphocyte counts and a sustained drop of lymphocyte GSH levels towards normal. Two patients (#2, #7) showed stabilization of the tumor, increased haemoglobin levels. In three patients (#4, #5, #6) the disease progressed with a trend toward higher lymphocyte GSH levels. These results indicate that whey protein concentrate might deplete tumor cells of GSH and render them more vulnerable to chemotherapy.

PRESENTED AT THE INTERNATIONAL CONFERENCE ON AIDS; INT. CONF. AIDS AUG. 7-12, 1994 (Abstract no. 421A).

42-ANTI-HIV AND ANTI-APOPTOTIC ACTIVITY OF THE WHEY PROTEIN CONCENTRATE: *IMMUNOCAL.*

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OBJECTIVES: The in vivo glutathione (GSH) promoting activity of undenatured Whey protein concentrate (WPC) has already been demonstrated. Here we demonstrate the anti HIV and anti Apoptotic activity of a WPC product termed IMMUNOCAL and its relation with GSH synthesis.

METHODS: IMMUNOCAL is produced in linear fashion in order to maintain proteins in a non denatured form and to preserve their glutamyl cysteine residues. We tested the in vitro anti-HIV activity on cord blood mononuclear cells and MT 4 cells by studying each of reverse transcriptase (RT) activity, p24 antigen production, and syncytium formation. GSH was measured by spectrophotometric recycling assay. Apoptosis was evaluated by flow cytometry on PBMC from HIV infected individuals (cells were stained with acridine orange and ethidium bromide) (n = 6).

RESULTS: An anti HIV activity was found at WPC concentrations between 100 micrograms/ml and 500 micrograms/ml. Inhibition of syncytium formation occurred with a IC50 of 150 micrograms/ml. PBMCs cultured with these WPC concentrations (N = 3) had a statistically significant increase in GSH synthesis when compared to untreated cells, 9.6 +/- 1.5 vs 5.4 +/- nmoles/10(7) cells, p = 0.01. HIV infected PBMCs cultured in the presence of 100 micrograms/ml of WPC were less prone to die of apoptosis than untreated cells, 15% +/- 2.6 vs 37% +/- 2.4 p <0.001.

CONCLUSION: IMMUNOCAL (WPC) possesses antiviral and anti-apoptotic activities which may be related to its glutathione promoting activity. A clinical trial is currently going on with children with AIDS and wasting syndrome.

OXIDATIVE STRESS, CELL ACTIVATION AND VIRAL INFECTION • C. PASQUIER ET AL. (EDS) • ©1994 BIRKHÄUSER VERLAG BASEL/SWITZERLAND

43-PLACE FOR AN ANTIOXIDANT THERAPY IN HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

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SUMMARY - Oxidative stress, a known activator of HIV replication in vitro, has a potential role as a cofactor of HIV disease progression. Arguments supporting the role of oxidative stress as a cofactor in HIV activation are summarized in this review. The role of intracellular antioxidants such as glutathione (GSH), and drugs and nutriceutical agents promoting GSH synthesis, are discussed. The review also includes the early results of nutritional interventions based on a diet enriched with IMMUNOCAL™, a whey protein concentrate prepared in a proprietary manner.

CLIN INVEST MED, 16:204-209, 1993.

44-WHEY PROTEINS AS A FOOD SUPPLEMENT IN HIV-SEROPOSITIVE INDIVIDUALS

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ABSTRACT – On the basis of numerous animal experiments, a pilot study was undertaken to evaluate the effect of undenatured, biologically active, dietary whey protein in 3 HIV-seropositive individuals over a period of 3 months. Whey protein concentrate was prepared so that the most thermosensitive proteins, such as serum albumin which contains 6 glutamylcysteine groups, would be in undenatured form. Whey protein powder dissolved in a drink of the patient's choice was drunk cold in quantities that were increased progressively from 8.4 to 39.2 g per day. Patients took whey proteins without adverse side effects. In the 3 patients whose body weight had been stable in the preceding 2 months, weight gain increased progressively between 2 and 7 kg, with 2 of the patients reaching ideal body weight. Serum proteins, including albumin, remained unchanged and within normal range, indicating that protein replenishment per se was not likely the cause of increased body weight. The glutathione content of the blood mononuclear cells was, as expected, below normal values in all patients at the beginning of the study. Over the 3-month period, GSH levels increased and in one case rose by 70% to reach normal value. The increase in body weight observed in these patients did not correlate with increase in energy or protein intake.

In conclusion, these preliminary data indicate that, in patients who maintain an adequate total caloric intake, the addition of "bioactive" whey protein concentrate as a significant portion of total protein intake increases body weight and shows elevation of glutathione (GSH) content of mononuclear cells toward normal levels. This pilot study will serve as a basis for a much larger clinical trial.

CLIN INVEST MED, *14:* 296-309, 1991.

45-THE BIOLOGICAL ACTIVITY OF UNDENATURED DIETARY WHEY PROTEINS: ROLE OF GLUTATHIONE

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ABSTRACT – This study compared the effects of different sources of whey protein concentrate (20 g/100 g diet) and of casein on the spleen, liver, and heart glutathione content of C3H/HeJ mice, and on the immune response of their spleen cells to sheep red blood cells. Body weight curves were similar in all dietary groups. Our data indicate that the humoral immune response is highest in mice fed a dietary whey protein concentrate exhibiting the highest solubility (undenatured conformation) and a greater relative concentration of the thermolabile cystine rich proteins. In addition, the mice fed this type of whey protein concentrate exhibit higher levels of tissue glutathione. The presence in the serum albumin fraction of glutamylcysteine groups (rare in food protein) and the specific intramolecular bond as related to the undenatured conformation of the molecule are considered to be key factors in the glutathione-promoting activity of the protein mixture.

CANCER LETTERS, *57*: 91-94, 1991.

46-WHEY PROTEINS IN CANCER PREVENTION

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ABSTRACT – Epidemiological and experimental studies suggest that dietary milk products may exert an inhibitory effect on the development of several types of tumors. Some recent experiments in rodents indicate that the antitumor activity of the dairy products is in the protein fraction and more specifically in the whey protein component of milk. We and others have demonstrated that whey protein diets result in increased glutathione (GSH) concentration in a number of tissues, and that some of the beneficial effects of whey protein intake are abrogated by inhibition of GSH synthesis. Whey protein is particularly rich in substrates for GSH synthesis. We suggest that whey protein may be exerting its effect on carcinogenesis by enhancing GSH concentration.

TUMOR BIOL *11*: 129-136, 1990.

47-DIETARY MILK PROTEINS INHIBIT THE DEVELOPMENT OF DIMETHYLHYDRAZINE-INDUCED MALIGNANCY

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ABSTRACT – This study investigated the influence of two formula diets containing 20 g/100 g diet of either whey protein concentrate or casein, or Purina mouse chow on 1,2dimethylhydrazine (DMH)-induced colon carcinoma in A/J mice. Four weeks after the 24th DMH treatment the incidence of tumour and tumour area in the whey protein-fed mice was substantially less in comparison to either the casein or Purina groups. The Purina group exhibited the greatest tumour burden. At the end of the experiment all animals continuously fed the whey protein diet were found to be alive, whereas 33% of those on the casein or Purina diet had died. Animals fed Purina diet for 20 weeks and then switched to either milk protein diet for a further 8 weeks exhibited a decrease in tumour burden as compared to those animals fed the Purina diet continuously. Body weights were similar in all dietary groups. In conclusion, a whey protein diet appears to significantly influence the development of chemically induced colon tumours and the short-term survival of mice.

MINERVA DIETOL GASTROENTEROL *35(4)*: 241-5, 1989.

48-CHANGES IN BILIARY SECRETORY IMMUNOGLOBULINS A IN MICE FED WHEY PROTEINS

Costantino AM, Balzola F, Bounous G.
Article in Italian.

A whey protein diet has been shown to enhance splenic immune response to sheep red blood cells (SBRC) in mice. This study was designed to investigate the influence of the type of dietary protein on the biliary secretory IgA. A/J mice were fed defined formula diets containing either 20% whey protein, or 20% casein. Another group was fed Purina mouse chow. After 3 weeks of dietary treatment the body weight of each mouse was recorded and the gall-bladder was removed and its whole content analyzed by ELISA to determine S-IgA secretion. Body weight curves were similar in all dietary groups; higher biliary levels of S-IgA appeared in the whey protein fed mice than in the casein (p less than 0.025) or purine (p less than 0.025) fed mice. Dietary protein type may have a direct influence on the immune response in the gastrointestinal tract, without affecting body weight.

CLIN INVEST MED, *12:* 343-349, 1989.

49-THE INFLUENCE OF DIETARY WHEY PROTEIN ON TISSUE GLUTATHIONE AND THE DISEASES OF AGING

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ABSTRACT – This study compared the effects of a whey-rich diet (20 g / 100 g diet), with that of Purina mouse chow or casein-rich diet (20 g / 100 g diet), on the liver and heart glutathione content and on the survival of old male C57BL / 6 NIA mice. The study was performed during a limited observation period of 6.3 months. In mice fed the whey protein-rich diet between 17 months and 20 months of age, the heart tissue and liver tissue glutathione content were enhanced above the corresponding values of the casein diet-fed and Purina-fed mice. Mice fed the whey protein diet at the onset of senescence, exhibited increased longevity as compared to mice fed Purina mouse chow over the 6.3 month observation period extending from the age of 21 months (corresponding to a human age of 55 years) to 26-27 months of age (corresponding to a human age of 80 years), during which time 55% mortality was observed. The corresponding mean survival time of mice fed the defined casein diet is almost identical to that of Purina-fed controls. Body weight curves were similar in all three dietary groups. Hence, a whey protein diet appears to enhance the liver and heart glutathione concentration in aging mice and to increase longevity over a 6.3 month observation period.

CLIN INVEST MED, *12:* 154-61, 1989.

50-IMMUNOENHANCING PROPERTY OF DIETARY WHEY PROTEIN IN MICE: ROLE OF GLUTATHIONE

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ABSTRACT – The spleen cells immune response to sheep red blood cells of C3H/HeJ mice fed a 20 g whey protein/100 g diet is substantially higher than that of mice fed an equivalent casein diet of similar nutritional efficiency. The present study indicates that the observed immunoenhancing effect of the whey protein mixture is dependent on the overall amino acid pattern resulting from the contribution of all its protein components. Whey protein contains substantially more cysteine than casein. Dietary cysteine is considered to be a rate limiting substrate for the synthesis of glutathione which is necessary for lymphocyte proliferation. Our studies show that enhancement of host humoral immune response is associated with greater and more sustained production of splenic glutathione during the antigen driven clonal expansion of the lymphocyte in whey protein fed mice in comparison to mice fed the equivalent casein or the cysteine-enriched casein diet. Hence the efficiency of dietary cysteine in inducing supernormal glutathione levels is greater when it is delivered in the whey protein than as free cysteine. Administration of S-(n-butyl) homocysteine sulfoximine, which reduces splenic glutathione level by half, produces a 4-5 fold drop in the humoral immune response of whey protein diet-fed mice. This is further evidence of the important role of glutathione in the immunoenhancing effect of dietary whey protein.

CLINICAL AND INVESTIGATIVE MEDICINE, VOL. 11,.NO. 4,.PP 271-278,. 1988.

51-THE IMMUNOENHANCING PROPERTY OF DIETARY WHEY PROTEIN CONCENTRATE

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(ORIGINAL MANUSCRIPT SUBMITTED OCTOBER 22, 1987: ACCEPTED IN REVISED FORM JANUARY 25, 1988)

ABSTRACT - The plaque-forming cell response to sheep red blood cells was found to be enhanced in mice fed a formula diet containing 20 g lactalbumin /100 g diet in comparison to mice fed equivalent formula diets of similar nutritional efficiency containing 20 g / 100 g diet of either casein, soy, wheat or corn protein, egg albumin, beef or fish protein, *Spirulina maxima,* or *Scenedesmus protein*, or Purina mouse chow. This effect was manifest after 2 weeks and persisted for at least 8 weeks of dietary treatment. Mixing lactalbumin with either casein or soy protein in a 20 g protein / 100 g diet formula significantly enhanced the immune response in comparison to that of mice fed diets containing 20% soy protein or casein.

key words: dietary whey protein, humoral immune response.

CLIN INV MED, *11*: 213-217, 1988.

52-DIETARY WHEY PROTEIN INHIBITS THE DEVELOPMENT OF DIMETHYLHYDRAZINE-INDUCED MALIGNANCY

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ABSTRACT – This study investigates the influence of two formula diets containing 20 g/100 g diet of either whey protein concentrate or casein or Purina mouse chow, on the humoral immune responsiveness and dimethylhydrazine induced colon carcinogenesis in A/J mice. After 20 weeks of dimethylhydrazine treatment, the number of plaque forming cells per spleen, following intravenous inoculation with 5 x 10^6 sheep red blood cells, was nearly three times greater in the whey protein-fed group than in the casein-fed mice although both values were substantially below normal. After 24 weeks of dimethylhydrazine treatment the incidence of tumors in the whey protein-fed mice was substantially lower than that in mice fed either the casein or Purina diet. Similarly, the tumor area was less in the whey protein group in comparison to either the casein or Purina groups, with some difference between casein and Purina groups. Body weight curves were similar in all dietary groups.

In conclusion, a whey protein diet appears to significantly inhibit the incidence and growth of chemically induced colon tumors in mice.

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53-GLUTATHIONE AUGMENTS THE ACTIVATION OF CYTOTOXIC T LYMPHOCYTES *IN VIVO*

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ABSTRACT – The activation of cytotoxic T lymphocytes (CTL) *in vivo* was found to be augmented by glutathione if injected i.p. in the late phase but not in the early phase of the response. The effect of glutathione possibly resembles the augmenting effect of 2-mercaptoethanol in lymphocyte cultures.

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54-MECHANISM OF ALTERED B-CELL RESPONSE INDUCED BY CHANGES IN DIETARY PROTEIN TYPE IN MICE

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ABSTRACT – The effect of 20 g/100 g dietary lactalbumin (L) or casein (C) diets or a nonpurified (NP) diet on the immune responsiveness of C57B1/6J, C3H/HeJ and BALB/cJ mice has been investigated by measuring the response to the T cell-independent antigen, TNP-Ficoll. To investigate the possible influence of dietary protein type on the supply of B lymphocytes, bone marrow lymphocyte production has been examined by a radioautographic assay of small lymphocyte renewal and an immuno-fluorescent stathmokinetic assay of pre-B cells and their proliferation. The humoral response of all mice fed the L diet was found to be higher than that of mice fed the C diet or non purified diet. A similar pattern of dietary protein effect in (CBA/N x DBA/2J) F_1 mice carrying the *xid* defect was observed following challenge with sheep red blood cells (SRBC). An even greater enhancing effect of dietary L was noted in normal (DBA/2J x CBA/N) F_1 mice after immunization with SRBC, but in contrast, the normal large-scale production of B lymphocytes in mouse bone marrow was independent of the type of dietary protein. Dietary protein type did not affect blood level of minerals and trace metals. The free plasma amino acid profile essentially conformed to the amino acid composition of the ingested protein, suggesting that the changes in plasma amino acid profile might be a crucial factor in diet-dependent enhancement or depression of the B-cell response. The findings indicate that the observed effects of altered dietary protein type on humoral immune responsiveness are not exerted centrally on the rate of primary B-lymphocyte production in the bone marrow, but may reflect changes either in the functional responsiveness of the B lymphocytes themselves or in the processes leading to their activation and differentiation in the peripheral lymphoid tissues.

INDEXING KEY WORDS: DIET – PROTEIN – IMMUNITY – B-CELL RESPONSE - MICE

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55-DIFFERENTIAL EFFECT OF DIETARY PROTEIN TYPE ON THE B-CELL AND T-CELL IMMUNE RESPONSES IN MICE

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ABSTRACT – The effect of 20 g/100 g diet of lactalbumin (L), casein (C), soy (S) and wheat (W) protein on the immune responsiveness of C3H/HeN mice has been investigated by measuring the humoral immune response to the T cell-independent antigen, TNP-Ficoll. The humoral immune response of mice fed the L diet was found to be higher than that of mice fed the C, S and W diets. On the other hand, delayed-type hypersensitivity, and splenic cell mitogen responses to phytohemagglutinin and concanavalin A did not differ among mice fed

the various diets. Similarly, the type of diet did not appear to influence host resistance to *Salmonella typhymurium*. It is postulated that the type of protein in the diet influences directly the intrinsic capacity of the B lymphocytes to respond to an immunogenic stimulus.

Indexing Key Words: diet * protein * immunity * mice

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56-INFLUENCE OF DIETARY PROTEIN TYPE ON THE IMMUNE SYSTEM OF MICE

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ABSTRACT – The effect of graded amounts of dietary lactalbumin (L), casein (C), soy (S), wheat (W) protein and Purina rodent chow (stock diet) on the immune responsiveness of C3H/HeN mice has been investigated by measuring the specific humoral immune response to sheep red blood cells (SRBC), and horse red blood cells (HRBC) as well as the nonspecific splenic cell responsiveness to phyto-hemagglutinin (PHA) and concanavalin A (Con A) after stimulation with *Myco-bacterium bovis*, strain BCG. The nutritional efficiency of these diets was normal and similar. The immune response of mice fed the L diets, was found to be almost five times higher than that of mice fed the corresponding C diets. The humoral immune response of mice fed C, S, and W diets was substantially lower than that of mice fed stock diet, whereas that of mice fed L diet was higher. The above-described immune effect of all tested proteins was obtained at 20 g/100 g concentration with no further increments with 30- and 40 g/100 g protein in the diet. Mitogen responsiveness to PHA and Con A in L diet-fed mice was only slightly higher than that of C diet-fed mice. Little difference in immune responses was noted among mice fed C, S or W protein diets. The principal factor responsible for the observed immune effect does not appear to be the availability or concentration of single essential amino acids but rather the composite effect of the specific amino acid distribution in the protein.

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57-INFLUENCE OF DIETARY PROTEINS ON THE IMMUNE SYSTEM OF MICE

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ABSTRACT The effect of graded amounts of dietary laetalbumin (L) and casein (C) hydrolyzates on the immune responsiveness of C3H/HeN and DBA/2 strain mice has been investigated by measuring both the specific humoral immune response to sheep red blood cells (SRBC) and the nonspecific splenic cell responsiveness to phytohemagglutinin, concanavalin A and *Escherichia coli* lipopolysaccharide after stimulation with *Mycobacteri*urn *bovis,* strain BCG. The nutritional efficiency of these diets was similar at both 12 and 28% amino acid levels. The immune responses of mice fed the L diets were found to be significantly greater than those of mice fed the corresponding C diets, especially at the 28% level. Furthermore in the mice fed L diet, increasing the concentration of amino acid in the diet from 12 to 28% greatly enhanced immune responsiveness by both parameters measured. In the C-fed mice, a comparable enhancement of mitogen responsiveness with increasing amino acid level of diet was seen, but there was no change in the humoral immune response. The enhancement of immune responsiveness observed in mice fed the 28% L diet was moderately reduced by the addition of phenylalanine to the diet, indicating that the lower level of this amino acid in the L protein may be of some significance. These dietary effects on immune responsiveness were remarkably similar in both mouse strains tested.

INDEXING KEY WORDS: diet - protein – immunity - mice

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58-INFLUENCE OF DIETARY LACTALBUMIN HYDROLYSATE ON THE IMMUNE SYSTEM OF MICE AND RESISTANCE TO SALMONELLOSIS

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ABSTRACT – In the present study we investigated the effect of four weeks of treatment with a diet containing lactalbumin hydrolysate (LAH: Nestlé, Vevey, Switzerland) on the immune response of C3H/HeN mice. Our data indicate that it was possible to increase the level of this type of protein in the diet above the minimum requirement (12% LAH) and thus produce augmented humoral immune responsiveness and resistance to salmonellosis.
Lactalbumin = Whey Protein Concentrate