

The Influence Of Dietary Whey Protein On Tissue Glutathione And The Diseases Of Aging

Gustavo Bounous^{1,2}, Francine Gervais^{1,3}, Victor Amer^{1,3}, Gerald Batist³, and Phil Gold^{1,3}

The Montreal General Hospital Research Institute¹ and McGill University, Departments of Surgery², and Medicine³

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.

Immunoenhancing Property Of Dietary Whey Protein In Mice: Role Of Glutathione.

G. Bounous, G. Batist, P. Gold

Montreal General Hospital, Quebec

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.

The Biological Activity Of Undenatured Dietary Whey Proteins: Role Of Glutathione.

G. Bounous, P. Gold

Department of Surgery, Montreal General Hospital, Research Institute, Quebec

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.

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

The Effect Of Supplementation With A Cysteine Donor On Muscular Performance

LC Lands, MD, PhD*†, VL Grey, PhD†‡, AA Smountas, BSc*

**Division of Respiratory Medicine, † Department of Pediatrics, ‡Department of Biochemistry, McGill University Health Centre-Montreal Children's Hospital, Montreal, Quebec, Canada*

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

Treatment Of Obstructive Airway Disease With A Cysteine Donor Protein Supplement: A Case Report

Bryce Lothian, MD*, Vijaylaxmi Grey, PhD*†, R. John Kimoff, MD‡, Larry Lands, MD, PhD*§

**Department of Pediatrics, †Department of Biochemistry, §Division of Respiratory Medicine, McGill University Health Centre-Montreal Children's Hospital, Montreal, Quebec, Canada*

‡Division of Respiratory Medicine, McGill University Health Centre-Royal Victoria Hospita, Montreal, Quebec, Canada

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.

Dietary Whey Protein Inhibits the Development of Dimethylhydrazine-Induced Malignancy

G. Bounous*, R. Papenburg*, P.A.L Kongshavn**, P. Gold†, and D. Fleiszer*

Departments of Surgery, Physiology**, and Medicine†, Montreal General Hospital and McGill University*

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 5×10^6 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.

TUMOR BIOL 11: 129-136, 1990

Dietary Milk Proteins Inhibit the Development of Dimethylhydrazine-Induced Malignancy¹

R. Papenburg^a, G. Bounous^a, D. Fleiszer^a, P. Gold^b

Departments of ^aSurgery and ^bMedicine, The Montreal General Hospital and McGill University, Montreal, Quebec, Canada

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.

¹ This work was supported by grants from the Medical Research Council of Canada, and The Dairy Bureau of Canada.

Whey Proteins In Cancer Prevention

G. Bounous*, G. Batist** and P. Gold***

*Professor of Surgery, McGill University, and Career Investigator of the Medical Research Council of Canada,
Director, Experimental Therapeutics, Department of Oncology, McGill University, *Chairman, Department of
Medicine, McGill University, and Physician-in-Chief, The Montreal General Hospital.

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.

Anticancer RESEARCH 15: 2643-2650, 1995

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

RENEE S. KENNEDY¹, GEORGE P. KONOK¹, GUSTAVO BOUNOUS², SYLVAIN BARUCHEL³
and TIMOTHY D.G. LEE⁴

¹Department of Surgery, Dalhousie University, Halifax, Nova Scotia:

²Department of Surgery, McGill University, Montreal Quebec

³Department of Pediatrics and Oncology, McGill University, Montreal, Quebec:

⁴Department of Immunology and Microbiology, Dalhousie University, Halifax, Nova Scotia, Canada

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.

Influence Of Dietary Lactalbumin Hydrolysate On The Immune System Of Mice And Resistance To Salmonellosis

G. Bounous, M.M. Stevenson*, P.A.L. Kongshavn†

*Centre hospitalier universitaire, Sherbrooke, Quebec, Canada; *Montreal General Hospital Research Institute and †McGill University, Montreal, Quebec, Canada*

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

J. NUTR. 113: 1415-1421, 1983

Influence Of Dietary Protein Type On The Immune System Of Mice²

G. Bounous,³ L. Létourneau and P.A.L. Kongshavn†

Centre hospitalier universitaire, Sherbrooke, Quebec, Canada; J1H 5N4 and †Montreal General Hospital Research Institute and Department of Physiology, McGill University, Montreal, Quebec, Canada, H3G 1Y6

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 *Mycobacterium 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.

LACTALBUMIN = WHEY PROTEIN CONCENTRATE

² Supported by grants from the Medical Research Council of Canada.

³ Dr. G. Bounous is a career investigator of the Medical Research Council of Canada.

Mechanism Of Altered B-Cell Response Induced By Changes In Dietary Protein Type In Mice⁴

G. Bounous, N. Shenouda,* P.A.L. Kongshavn† and D.G. Osmond*

*Department of Surgery, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada, J1H 5N4; *Department of Anatomy, McGill University, Montreal, Quebec, Canada, H3A 2B2; and †Department of Physiology, McGill University, Montreal, Quebec, Canada, H3A 2B2*

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 nonpurified diet. A similar pattern of dietary protein effect in (CBA/N x DBA/2J) F₁ 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) F₁ 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

LACTALBUMIN = WHEY PROTEIN CONCENTRATE

⁴ Supported by grants from the Medical Research Council of Canada and from the National Cancer Institute of Canada.

Whey Proteins As A Food Supplement In HIV-Seropositive Individuals

G. Bounous, S. Baruchel, J. Falutz, P. Gold

Departments of Surgery and Medicine, The Montreal General Hospital and McGill University, Montreal, Quebec

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.

Immuno-Enhancing Properties Of Undenatured Milk Serum Protein Isolate In HIV Patients.

G. Bounous

Former Professor, Department of Surgery, McGill University and Career Investigator of the Medical Research Council of Canada

ABSTRACT – Cellular glutathione (GSH) is a tightly self-regulated system because of the feedback inhibition of γ -glutamylcysteine synthetase activity by GSH level. However, when GSH is depleted, as in the lymphocytes of mice during the immune response or in the lymphocyte of AIDS patients, the cysteine delivery system in Immunocal produces a substantial increase in cellular GSH up to, but not above, normal values. Preliminary data in AIDS patients demonstrate that this is associated with major improvements in health.

These clinical data and the *in vitro* demonstration that Immunocal inhibits the HIV virus while increasing GSH synthesis strongly suggest that an antagonistic relation exists between the virus and cellular GSH.

Unlike specific antiretroviral drugs which may induce mutation, hence resistance of the virus to therapy, the normalization of the lymphocyte glutathione levels and redox status through a cysteine delivery system represents a totally different approach by which the natural cellular defense system is boosted.

Two case reports involving a mother and son are worthy of note. A young HIV positive lady could not tolerate the headache caused by AZT treatment. She was then given Immunocal (20g a day), and followed for a 7-month period. Her

lymphadenopathy cleared and had not reappeared since; her viral load gradually dropped from 19.000 copies/mL to 3.000. Only slight increase in blood WBC. Her two year old son was tested positive about the same time as the parents. Because of the unbearable side effect of antiretroviral therapy, experienced by the mother, the son was given only Immunocal (10g daily). His viral load steadily dropped from 140.000 copies m/L to 5.000 and his blood lymphocyte increased from 2.5 K/c mm to 3.33, the neutrophils from 1.6 to 3.65 and CD4 from 1025 to 1450 over the 7-month period. What is particularly interesting about this case is the following events: the young mother became pregnant and for the sake of the fetus she accepted to receive AZT while on Immunocal. To her surprise, this time, she did not suffer the side effects of the drug. However, she developed severe post partum depression for which she was hospitalized. The treating psychiatrist forced her to stop the whey protein intake and soon after, she experienced the typical dreadful AZT related headache. The headache ended with the resumption of Immunocal treatment. It may be relevant that it was recently determined that there is a direct relationship between mitochondrial DNA damage and oxidation of mitochondrial GSH by AZT. It is conceivable that GSH restoration by whey protein could prevent to a certain extent the adverse effect of AZT.

Nutriceutical Modulation Of Glutathione With A Humanized Native Milk Serum Protein Isolate, Immunocal™: Application In AIDS And Cancer.

S. Baruchel*, G. Viau*, R. Olivier**, G. Bounous***, M.A. Wainberg****

McGill University – Montreal Children's Hospital Research Institute, Montreal, Quebec, Canada, **Pasteur Institute Paris, France, *Montreal General Hospital, Montreal, Quebec, Canada, ****Jewish General Hospital, Lady Davis Institute, Montreal, Quebec, Canada.*

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.