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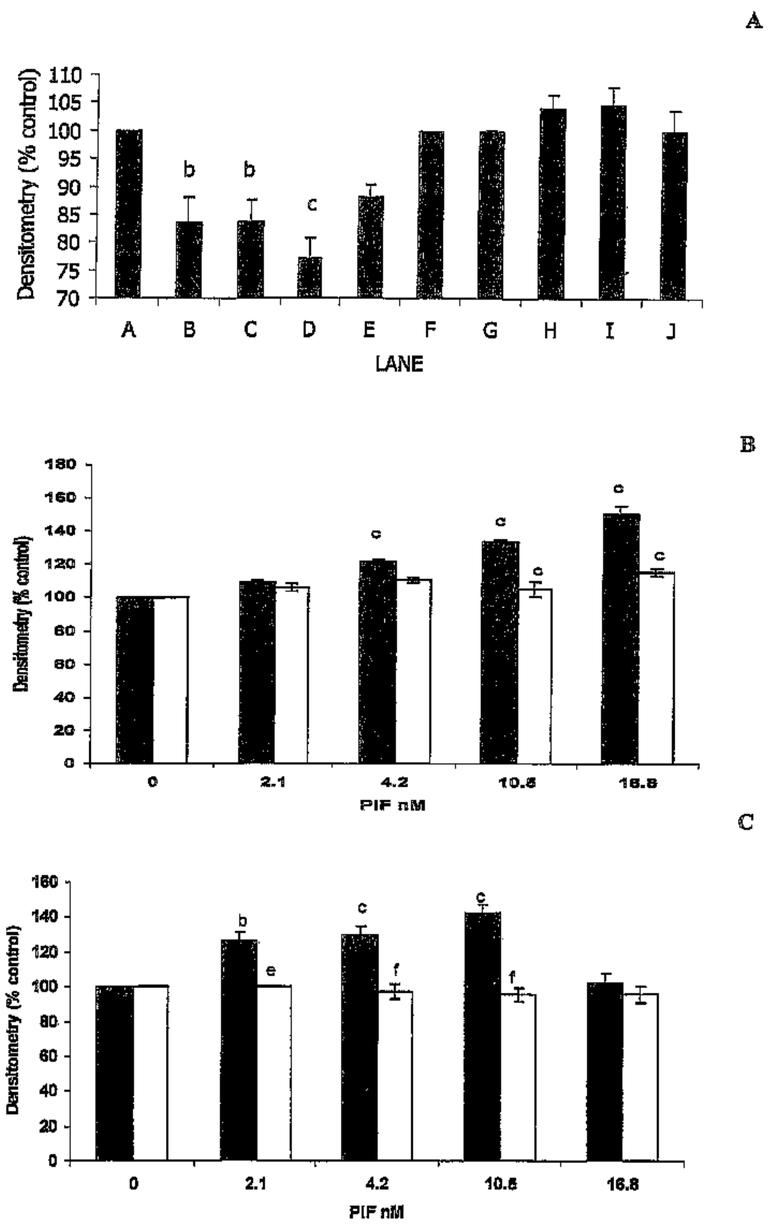
(54) Titre : COMPOSITIONS DE HMB ET LEURS UTILISATIONS
(54) Title: HMB COMPOSITIONS AND USES THEREOF



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(54) Title: HMB COMPOSITIONS AND USES THEREOF

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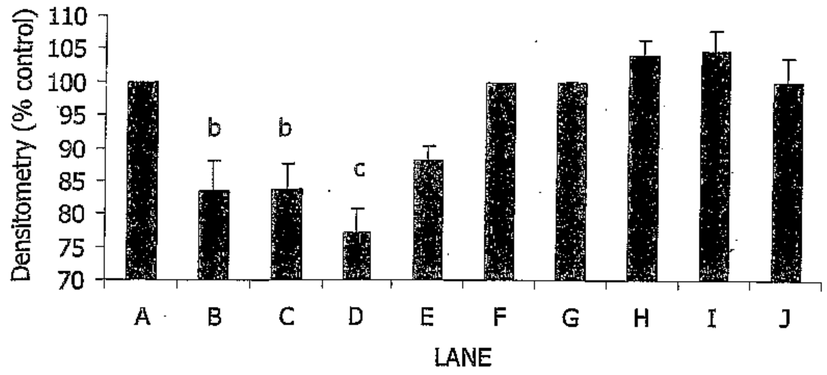

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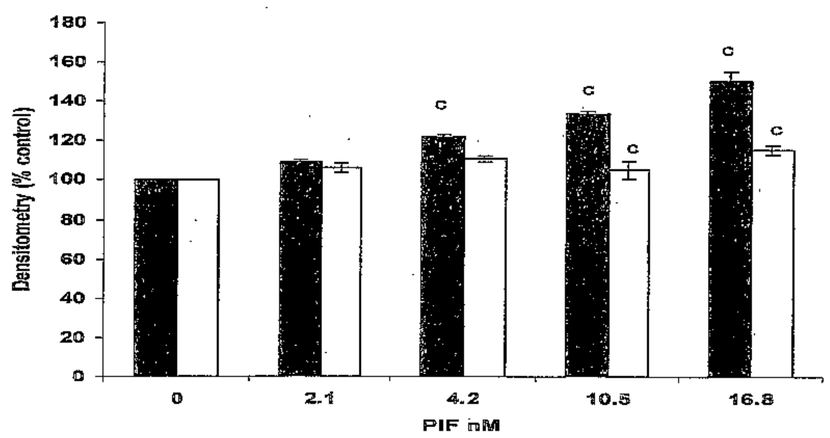

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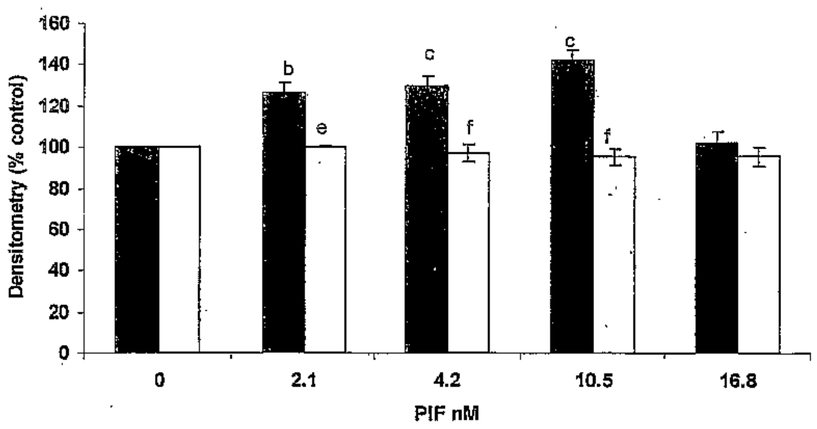

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HMB COMPOSITIONS AND USES THEREOF

The present invention relates to methods for the prevention and treatment of chronic inflammatory diseases, cancer, and involuntary weight loss. In the practice of the present invention patients are enterally administered HMB alone or alternatively in combination with eicosapentaenoic (20:5 ω–3), FOS, carnitine and mixtures thereof. HMB may be added to food products comprising a source of amino-nitrogen enriched with large neutral amino acids such as leucine, isoleucine, valine, tyrosine, threonine and phenylalanine and subtantially lacking in free amino acids.

BACKGROUND

Undesired weight loss, particularly lean mass loss is a relatively common occurance in critical illness, and has a significant impact on morbidity and mortality. This is particularly true in cancer patients, where such mass losses can become tretment-limiting, and thus impact overall prognosis.

Cachexia is a syndrome characterized by anorexia, weight loss, premature satiety, asthenia, loss of lean body mass, and multiple organ dysfunction. It is a common consequence of chronic illnesses (both malignant and non-malignant) and is associated with a poorer prognosis in chronic obstructive pulmonary disease (COPD), chronic heart failure (CHF), renal failure, AIDS, dementia, chronic liver disease and cancer. It is often independent of other indicators of disease severity. (Witte, K. K. A. and Clark, A. L.: Nutritional abnormalities contributing to cachexia in chronic illness., International Journal of Cardiology 85:23-31, 2002)

Pulmonary disease is often associated with cachexia, and substantial numbers of patients suffering from COPD, particularly emphysema, become emaciated during the course of the disease. Weight loss is an independent risk factor for prognosis, and is often associated with increased oxygen consumption. This has been linked with development of inefficient muscle energy metabolism (Kutsuzawa, T, et al.: Muscle energy metabolism and nutritional status in patients with chronic obstructive pulmonary disease. Am. J. Respir. Crit. Care Med. 152(2):647-652, 1995). COPD is also associated with a general elevated systemic inflammatory response, reflected by elevated concentrations of pro-inflammatory cytokines and acute phase proteins in the peripheral blood (Schols, A. M., et al: Evidence for a relation between metabolic derangements and increased levels of inflammatory mediators in a subgroup of patients with chronic obstructive pulmonary disease. Thorax 51:819-824, 1996; Takabatake, N., et al.: Circulating leptin in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 159:1215-1219,

1999; Dentener, M. A., et al.: Systemic anti-inflammatory mediators in COPD: increase in soluble interleukin I receptor II during treatment of exacerbations. Thorax 56:721-726, 2001.) Such changes are often associated with muscle wasting syndromes.

Studies with incubated muscles and muscle extracts suggest that the ATP-dependent ubiquitin-proteosome pathway is responsible for most of the increased proteolysis which ultimately results in muscle wasting. In particular, increased levels of ubiquitin-conjugated proteins, and increases in mRNA levels for polyubiquitin, certain proteosome subunits and the ubiquitin-conjugating enzyme $E2_{14K}$ are features found in most atrophying muscles (Schols, A. M. W. J.: Pulmonary cachexia. Intl J Cardiology 85:101-110, 2002; Jagoe, R. T. and Goldberg, A. L.: What do we really know about the ubiquitin-proteosome pathway in muscle atrophy? Curr Opin Clin Nutr Metab Care 4:183-190, 2001).

The majority of patients with cancer whose disease progresses to metastatic disease develop cachexia during their treatment program and the cachexia contributes to their deaths. The frequency of weight loss in cancer patients ranges from 40% for patients with breast cancer, acute myelocytic leukemia, and sarcoma to more than 80% in patients with carcinoma of the pancreas and stomach. About 60% of patients with carcinomas of the lung, colon or prostate have experienced weight loss prior to beginning chemotherapy. Although the relationship between pretreatment malnutrition (weight loss) and adverse outcome is established, no consistent relationship has been demonstrated between the development of cachexia and tumor size, disease stage, and type or duration of the malignancy.

Cancer cachexia is not simply a local effect of the tumor. Alterations in protein, fat, and carbohydrate metabolism occur commonly. For example, abnormalities in carbohydrate metabolism include increased rates of total glucose turnover, increased hepatic gluconeogenesis, glucose intolerance and elevated glucose levels. Increased lipolysis, increased free fatty acid and glycerol turnover, hyperlipidemia, and reduced lipoprotein lipase activity are frequently noted. The weight loss associated with cancer cachexia is caused not only by a reduction in body fat stores but also by a reduction in total body protein mass, with extensive skeletal muscle wasting. Increased protein turnover and poorly regulated amino acid oxidation may also be important. The presence of host-derived factors produced in response to the cancer have been implicated as causative agents of cachexia, e.g., tumor necrosis factor-α (TNF) or cachectin, interleukin-1 (IL-1), IL-6, gamma-interferon (IFN), and prostaglandins (PGs) (e.g., PGE_2).

Weight loss is common in patients with carcinomas of the lung and gastrointestinal tract, resulting in a massive loss of both body fat and muscle protein, while non-muscle

protein remains unaffected. While loss of body fat is important in terms of energy reserves, it is loss of skeletal muscle protein that results in immobility, and eventually impairment of respiratory muscle function, leading to death from hypostatic pneumonia. Although cachexia is frequently accompanied by anorexia, nutritional supplementation alone is unable to maintain stable body weight and any weight that is gained is due to an increase in adipose tissue and water rather than lean body mass. The same is true for appetite stimulants, such as megestrol acetate and medroxyprogesterone acetate, suggesting that loss of lean body mass is due to factors other than energy insufficiency.

Skeletal muscle mass is a balance between the rate of protein synthesis and the rate of degradation. Patients with cancer cachexia show a depression of protein synthesis in skeletal muscle and an increase in protein degradation, which is reflected in an increased expression of the ubiquitin-proteasome proteolytic pathway, the major determinant of protein degradation. Thus skeletal muscle from cachectic cancer patients shows increased expression of mRNA for both ubiquitin and proteasome subunits, while proteasome proteolytic activity increased in parallel with ubiquitin expression. The inability of anabolic stimuli to increase lean body mass in cachectic patients suggests that protein degradation must be attenuated before muscle mass can increase. Eicosapentaenoic acid (EPA), downregulates the increased expression of the ubiquitin-proteasome proteolytic pathway in the skeletal muscle of cachectic mice, and has been shown to stabilize body weight in cachectic patients with pancreatic cancer. When patients consumed an energy-dense supplement containing 32g protein and 2g EPA body weight increased and this was attributed solely to an increase in lean body mass (Barber, M.D., Ross, J.A., Voss, A.C., Tisdale, M.J., Fearon, K.C.H. The effect of an oral nutritional supplement enriched with fish oil on weight-loss in patients with pancreatic cancer. Br. J. Cancer, 81: 80-86, 1999).

A recent study by May et al (May, P.E., Barber, A., D'Olimpio, J.T., Hourihane, A. and Abumrad, N.N. Reversal of cancer-related wasting using oral supplementation with a combination of β-hydroxy-β-methylbutyrate, arginine and glutamine. Am. J. Surg., 183: 471-479, 2002) showed a mixture of HMB, arginine and glutamine to be effective in increasing body weight in weight losing patients with advanced (stage IV) cancer. Moreover, the increase in body weight was attributed to an increase in fat-free mass, as observed with EPA.

The use of the polyunsaturated fatty acid eicosapentaenoic acid is suggested for the treatment of cachexia by inhibiting lipolytic activity of lipolytic agents in body fluids and the activity of the enzyme guanidino-benzoatase. See Tisdale, M.J., and Beck, A., U.S. Patent No. 5,457,130, issued October 10, 1995; and Tisdale, et al. *Cancer Research* 50:

5022-5026 (August 1990). However, the product taught by Tisdale was in a solid dosage form, requiring an already ill patient to swallow 12-16 capsules per day. This method had serious drawbacks, including difficulty in swallowing, belching, and bad odor.

HMB has been found to be useful within the context of a variety of applications. Specifically, in U.S. Pat. No. 6,031,000 to Nissen et al. describes a composition comprising from about 0.5 g to about 30 g of HMB, wherein from about 0.5 g to about 30 g is based on the weight of the calcium salt of HMB and from about 0.5 g to about 50 g of free L-arginine, and from about 0.5 g to about 50 g of free L-glutamine. This patent also provides a method for the treatment of disease-associated wasting of an animal, a method for decreasing the serum-level of triglycerides of an animal, a method for decreasing the serum viral load of an animal, and a method for redistributing fat in an animal having a visceral region and a subcutaneous region. All methods comprise administering to the animal a composition comprising HMB and at least one free amino acid.

U.S.Pat. No. 5,348,979 to Nissen et al. describes the use of HMB in the nitrogen retention in human subjects. The amount of HMB administered is effective to conserve protein as determined by reduction in urinary nitrogen. The method can be used with patients having a negative nitrogen balance due to disease conditions, and also with normal elderly persons who are subject to protein loss. The HMB may be administered orally or by intravenous infusion. An effective amount of HMB is within the range from 0.01 to 0.20 grams of HMB based on its calcium salt per kilogram body weight per 24 hours.

U.S. Pat. No. 5,028,440 to Nissen describes a method for raising meat producing domestic animals to increase lean tissue development. HMB or an edible salt thereof is administered to the animals in an amount for a sufficient length of time to obtain a substantial increase in lean tissue weight. The method is particularly adapted for use with ruminants, including beef cattle and lambs, since HMB is not subject to appreciable rumen destruction. The method can also be practiced with other domestic animals, including chickens, and turkeys. HMB is feed within the range of from 0.5 to 100 mg.

U.S. Pat. No. 4,992,470 to Nissen describes the use of HMB to be markedly more effective for activating the immune function of T lymphocytes of mammals than .alpha.-ketoisocaproate (KIC). For activation of the T lymphocytes, .HMB or an edible water-soluble salt thereof is administered to the mammal by a route through which the HMB enters the blood of the mammal. The amount administered is sufficient for effective enhancement of the blastogenesis of their T lymphocytes. The method is adapted for use

with domestic mammals, including particularly cattle, sheep, and swine. HMB can also be used with humans as an immune system stimulant. HMB (Ca-HMB basis) is orally or parenterally administered in an amount of 500 to 2,500 milligrams (mg) per human subject per 24 hours.

German patent DE 29707308 to Kunz describes the use of branched chain amino acids in combination with HMB to promote muscle generation in the weight training population. Kunz teaches that a supplement of 3 gm taken daily with a protein consumption of 200gm per day enhances the value of nutritional protein and significantly increases the protein efficiency. Kunz also teaches that better effects can be achieved when HMB is combined with protein hydrolysates and/or free amino aicd mixtures rather than with intact (pure) proteins.

US patent 5,976,50 to Engel et al. describes a dietary food supplement for weight reduction formed of a mixture of a sugar based confectionary containing therapeutic amounts of chitosan, kava and a fat burning nutriceutical which may include choline/inusital, chromium picolinate, HMB, carnitine and pyruvate. The nutriceutical ingredient mixed with the chitosan and kava functions to burn whatever fat the body has consumed, i.e. to metabolize better any fat that is ingested and not attracted to the chitosan.

Commercial products designed for the weight lifting population that contain HMB include Lean DynamX by EAS Inc. of Golden, Colorado. Lean DynamX provides a blend of ingredients that support fat loss without the use of strong stimulants. The ingredients include HMB, chromium picolinate, conjugated linoleic acid, mate leaves and stems and carnitine tartrate. The powder composition is mixed with water and taken 2-3 servings daily, with one serving taken 30 minutes before workouts.

Additional commercial products include Mega HMB Fuel® from Twinlab Corporation in Hauppauge, NY. Mega HMB Fuel® contains 750mg of HMB in one capsule. The suggested daily dosage is 4 capsules to support damage to muscle cells which can occur subsequent to intense resistance exercise.

Also of interest is U.S. Patent 5,444,054 to Garleb, et al. and a related U.S. Patent 5,780,451. These documents describe compositions and methods useful in the treatment of ulcerative colitis. Such compositions include a protein source that can be intact or hydrolyzed proteins of high biological value (col. 21); an indigestible oligosaccharide such as fructooligosaccharide; and a lipid blend containing a relatively high proportion of eicosapentaneoic acid, which contributes to a relatively high ω–3 to ω–6 fatty acid ratio.

Long chain fatty acid bio-pathways and physiological actions are discussed in U.S. Patent 5,223,285 to DeMichele, et al., the entirely of which is incorporated herein by reference.

The prevention and/or treatment of cachexia remain a frustrating problem. Both animal and human studies suggest that nutritional support is largely ineffective in repleting lean body mass in the cancer-bearing host. Randomized trials exploring the usefulness of total parenteral nutrition (TPN) support as an adjunct to cytotoxic antineoplastic therapy have demonstrated little improvement in treatment results. See for example Brennan, M.F., and Burt, M.E., 1981, *Cancer Treatment Reports* 65 (Suppl. 5): 67-68. This, along with a clear demonstration that TPN can stimulate tumor growth in animals suggests the routine use of TPN in cancer treatment is not justified. Kisner, D.L., 1981, *Cancer Treatment Reports* 65 (Suppl. 5): 1-2.

SUMMARY OF THE INVENTION

The present invention relates to methods for the prevention and treatment of chronic inflammatory diseases, cancer, and involuntary weight loss. In the practice of the present invention patients are enterally administered HMB alone or alternatively in combination with eicosapentaenoic (20:5 ω–3), FOS, carnitine and mixtures thereof.

In another embodiment, the present invention provides a method for the treatment of the disease-associated wasting of a patient. The method comprises administering to the patient the above-described composition, which comprises HMB in amounts sufficient to treat the disease-associated wasting, wherein, upon administration of the composition to the patient, the disease-associated wasting is treated.

In another embodiment, the present invention provides a method for reducing tumor growth rate in a patient. The method comprises administering to the patient the above-described composition, which comprises HMB in amounts sufficient to reduce tumor growth rate, wherein, upon administration of the composition to the patient, the tumor growth rate is reduced.

In another embodiment, the present invention provides a method for the prevention or treatment of diseases in patients by down regulating the expression and/or activity of protein kinase C, nuclear factor kappa-B, ubiquitin-conjugating enzymes, and components of 26S proteasome. These methods comprise administering to the patient HMB, its salts, metabolites or derivatives thereof.

In yet another embodiment, HMB may be added to food products comprising a source of amino-nitrogen enriched with large neutral amino acids such as leucine,

isoleucine, valine, tyrosine, threonine and phenylalanine and subtantially lacking in free amino acids.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure I presents a scheme describing the potential intracellular events in skeletal muscle involved in PIF induced proteasome activation.

Figure 2 presents dose-response curves for the effect of HMB on body weight (A) and tumor volume (B) in mice bearing the MAC16 tumor. HMB (in PBS) was administered orally by gavage on a daily regime at a concentration of 0.05 (●), 0.125 (○) and 0.25g/kg (X). Control mice received PBS alone (◆). The results shown are the mean ± SEM, where n=20.

Figure 3 presents the effect of HMB (0.25g/kg; ■), EPA (0.6g/kg; X) and the combination (○) together with PBS controls (●) on body weight of mice bearing the MAC16 tumor. Results shown are mean ± SEM, where n=20.

Figure 4 presents the weight of soleus muscles (A) and rate of protein degradation in soleus muscle (B) of mice bearing the MAC16 tumor and treated with either EPA (0.6g/kg), HMB (0.25g/kg) or the combination for 3 days. Values shown are mean ± SEM, where n=6.

Figure 5 presents the effect of HMB and EPA on proteasome functional activity, determined as the 'chymotrypsin-like' enzyme activity, in gastrocnemius muscle of mice bearing the MAC16 tumor and treated for 3 days. Results are shown as mean± SEM, where n=6.

Figure 6 presents the expression of proteasome 20S α-subunits (A) and β-subunits (B), detected by Western blotting, in gastrocnemius muscle of mice treated for 3 days with PBS (Control), HMB (0.25g/kg), EPA (0.6g/kg) or the combination. Densitometric analysis of the blots (n=6) are shown. A. control (closed bars), HMB (open bars), EPA (hashed bars) and combination (dotted bars)

Figure 7 presents the expression of proteasome 19S subunits, MSS1 (A) and p42 (B), detected by Western blotting, in gastrocnemius muscle of mice treated for 3 days with PBS (Control), HMB (0.25g/kg), EPA (0.6g/kg) or the combination (HMB+EPA). Densitometric analysis of the blots (n=6) are shown.

Figure 8 presents the expression of $E2_{14k}$, detected by Western blotting, in gastrocnemius muscle of mice treated for 3 days with PBS (Control), HMB (0.25g/kg), EPA

(0.6g/kg) or the combination (HMB+EPA). Densitometric analysis of the blots (n=6) are shown.

Figure 9 (A) presents the effect of PIF on total protein degradation in C_2C_{12} myotubes in the absence (X) or presence of either 50μM EPA (□), or 25μM (O) or 50μM (●) HMB. Measurements were made 24h after the addition of PIF and are shown as mean ± SEM, where n=9. 1(B) presents the chymotryptic activity of soluble extracts of murine myotubes treated with PIF in the absence or presence of EPA (50μM) or HMB (25 or 50μM). The symbols are the same as in (A). The results are shown as mean ± SEM, where n=9.

Figure 10 presents the effect of EPA and HMB on PIF-induction of 20S proteasome α-subunit **(A)**, β-subunit **(B)** and p42 **(C)**. The actin loading control is shown in **(D)**. Western blots of soluble extracts of C_2C_{12} myotubes 24h after treatment with PIF alone (lanes A-C) or with PIF in the presence of 50μM EPA (lanes D-F), 50μM HMB (lanes G-I) or 25μM HMB (lanes J-L) at a concentration of PIF of 4.2nM (lanes B, E, H and K) or 10nM (lanes C, F, I and L). Control cultures received PBS (lane A), 50μM EPA (lane D), 50μM HMB (lane G) or 25μM HMB (lane J). The blots shown are representative of three separate experiments.

Figure 11 presents the Western blot of the effect of PIF on cytoplasmic **(A)** and membrane-bound **(B)** PKC_α in murine myotubes. Cells were treated with PIF alone (lanes A-C) or with PIF in the presence of 50μM EPA (lanes D-F), 50μM HMB (lanes G-I) or 25μM HMB (lanes J-L) at 4.2nM (lanes B, E, H and K) or 10nM PIF (lanes C, F, I and L). Control cells received PBS (lane A), 50μM EPA (lane D), 50μM HMB (lane G) or 25μM HMB (lane J). The blots shown are representative of three separate experiments.

Figure 12 presents Western blots of total ERK 1/2 (p44 and p42) **(A)** and active (phosphorylated) ERK 1/2 **(B)** in soluble extracts of murine myotubes treated with PIF alone (lanes A-C) or with PIF in the presence of 50μM EPA (lanes D-F), 50μM HMB (lanes G-I) or 25μM HMB (lanes J-L) at a PIF concentration of 4.2nM (lanes B, E, H and K) or 10nM (lanes C, F, I and L). Control cells received either PBS (lane A), 50μM EPA (lane D), 50μM HMB (lane G) or 25μM HMB (lane J). The blots shown are representative of three separate experiments.

Figure 13 persents the effect of exposure of C_2C_{12} myotubes for 30min on cytosolic levels of IκBα **(A)**, determined by Western blotting, and activation of NF-κB binding to DNA, as determined by EMSA (**B** and **C**). The densitometric analysis is an average of 3 replicate blots or EMSAs. **(A)** Myotubes were treated with PIF alone (lanes A-E) or with PIF in the presence of 50μM HMB at a concentration of 0 (lanes A and F), 2.1 (lanes B and G), 4.2 (lanes C and H), 10.5 (lanes D and I) or 16.8nM PIF (lanes E and J). In **(B)** and **(C)** myotubes were treated with 0, 2.1, 4.2, 10.5 or 16.8nM PIF, in the absence (dark bars) or presence (open bars) of 25μM HMB **(B)** or 50μM HMB **(C)**.

DETAILED DESCRIPTION OF THE INVENTION

The term HMB, which is also referred to as beta-hydroxy-beta-methylbutyric acid, or beta-hydroxy-isovaleric acid, can be represented in its free acid form as $(CH_3)_2(OH)CCH_2$ COOH. HMB is a metabolite of leucine formed by transamination to alpha-ketoisocaproate (KIC) in muscle followed by oxidation of the KIC in the cytosol of the liver to give HMB.

The term "large neutral amino acids" refers to leucine, isoleucine, valine, tyrosine, threonine and phenylalanine. Amino acids are the building blocks of proteins. They are characterized by the presence of a carboxyl group (COOH) and an amino group (NH2) attached to the same carbon at the end of the compound.

The term "substantially lacking in free amino acids" refers to compositions which contain less than 0.4 grams of total free amino acid content in a daily dose of the composition. For example, if the product is designed to be fed at the rate of 1 can per day, then the one can of product contains less than a total of 0.4 grams of free amino acids. The amino acids in question are those naturally occuring L-isomers, consisting of one or more of the following compounds: L-alanine, L-arginine, L- asparagine, L-aspartic acid, L-cysteine (or L-cystine), L-glutamic acid, L-glutamine, glycine, L-histidine, L-Isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine, or their food- or pharmaceutically-acceptable salts, esters, salts or derivatives (such as methyl or ethyl esters).

The term "cachexia" refers to a state of general ill health and malnutrition. It is often associated with and induced by malignant cancer, and is characterized by loss of appetite, loss of body mass, especially lean body mass, and muscle wasting.

The term "fatty acids" refer to a family of carboxylic acids having a hydrocarbon chain, generally from about 12 to 24 carbons long. When unsaturated (having a double bond) at least one point in the hydrocarbon chain, such fatty acids are designated by the position of the first double bond. ω–3 fatty acids have a first double bond at the third carbon from the methyl end of the chain; and include, but are not limited to, α-linolenic

acid, stearidonic acid, eicosapentaenoic acid ("EPA"), docosapentaenoic acid and docosahexaenoic acid ("DHA") and the like. ω–6 fatty acids have a first double bond at the sixth carbon from the methyl end of the chain; and include, but are not limited to, linoleic acid, γ-linolenic acid, arachidonic acid ("AA"), and the like.

The term "food products" as used herein refer to delivery vehicles that contain one or more of fats, amino nitrogen and carbohydrates and provides some or all of the nutritional support for a patient in the recommended daily amounts. Frequently a food product will contain vitamins, minerals, trace minerals and the like to provide balanced nutrition to meal repalcements, medical foods, supplements. The food products may be in any typical form such as beverages, powders, bars, juices, carbonated beverages, bottled water.

The term "Reference Daily Intakes or RDI" refers to a set of dietary references based on the Recommended Dietary Allowances for essential vitamins and minerals. The Recommended Dietary Allowances are a set of estimated nutrient allowances established by the National Academy of Sciences, which are updated periodically to reflect current scientific knowledge.

The term "patient" refers to humans, dogs, cats, and any other non-ruminant animal.

Any reference to a numerical range in this application should be considered as being modified by the adjective "about". Further, any numerical range should be considered to provide support for a claim directed to a subset of that range. For example, a disclosure of a range of from 1 to 10 should be considered to provide support in the specification and claims to any subset in that range (i.e., ranges of 2-9, 3-6, 4-5, 2.2-3.6, 2.1-9.9, etc.).

While not intending the invention to be limited to any particular theory of operation, applicants describe below a probable mechanism.

In times of extreme need (e.g., starvation and the like), skeletal muscle is often used by the body as a reservior of amino acids and energy. This is mediated by upregulation of the proteolysis and downregulation of protein synthesis in muscle. The net result of which is release of amino acids from muscle to the general circulation for use in maintence of critical systems. When good health and adequate nutritient availability are restored, muscle is rebuilt. In the case of cachexia, this system is inappropriately activated, so even in the case of nutritional adequacy, muscle tissue proteins continue to be broken down.

One of the key proteolytic systems which are inappropriately activated is the ubiquitin proteosome system. When normally functioning, this system recognizes proteins

which are either aged or in some other manner either damaged or no longer needed, and marks them for removal via conjugation with ubiquitin. Such ubiquitinylated proteins are recognized by the proteosome, and degraded, releasing free ubiquitin and peptides and free amino acids in an energy - consuming process. There are a number of signaling molecules which activate or upregulate this system, including proteolysis - inducing factor (PIF), which is a protein factor produced by certain cachexia - inducing tumors. Binding of PIF to the muscle cell causes the upregulation of phospholipase A (PLA). This in turn produces signaling factors which ultimately activate protein kinase C, resulting in the activation of genes (via nuclear factor kappa B, NFκB) for ubiquitin conjugation and for certain subunits of the proteosome. The net result of all of this signaling is the up regulation of the ubiquitin proteosome system, and inappropriate, sustained protein degradation in the muscle. Figure 1 shows a detailed pathway of this activation sequence.

Protein Kinase C

Protein kinase C is a family of calcium – and lipid – activated serine-threonine kinases that play a key role in numerous intracellular signaling cascades. There are at least 12 different PKC isotypes, which are grouped into three classes based on their primary structure and biochemical properties (CA Carter: "Protein kinase C as a drug target: Implications for drug or diet prevention and treatment of cancer." Current Drug Targets 1:163-183 (2000). These are the conventional – (cPKCα, βI, βII and γ) which require diacylglycerol, phosphatidylserine and calcium for activation, novel (nPKCδ, ε, η, θ and μ) which require diacylglycerol and phosphatidylserine, but are calcium independent, and the atypical (aPKC λ, τ and ζ) which are calcium and diacylglycerol – independent.

PKC is synthesized as a membrane – bound proenzyme. Removal of the pro-sequence by proteolytic cleavage, and subsequent phosphorylation releases a competent enzyme from the membrane to the cytosol. Subsequent interaction with the peculiar sets of activators produces active enzyme. Thus, there are several levels of regulation possible, including control of expression, control of proteolytic processing, control of initial phosphorylation events and finally, regulation of the cytosolic levels of the various activators required for full activity.

Protein kinase C is involved in some of the signaling pathways leading to mitogenesis and proliferation of cells, apoptosis, platelet activation, remodelling of the actin cytoskeleton, modulation of ion channels and secretion. In addition, other observation that PKC is also the major receptor for tumor-promoting phorboly esters provided a key reagent for studying the mechanism of action of this enzyme. PKC regulates pathways relevant to inflammation, cardiovascular, peripheral microvascular,

CNS, oncology, immune and infectious disease states, and are considered as serious and important targets for drug development (P. G. Goekjian and M. R. Jirousek: "Protein Kinase C in the Treatment of Disease: Signal Transduction Pathways, Inhibitors, and Agents in Development" Current Medicinal Chemistry 6(9): 877 – 903, (1999); CA O'Brian, NE Ward, KR Gravitt and KP Gupta: "The tumor promoter receptor protein kinase C: A novel target for chemoprevention and therapy of human colon cancer." Growth Factors and Tumor Promotion: Implications for Risk Assessment, pages 117 – 120 © 1995, Wiley-Liss, Inc.; F Battaini: "Protein kinase C isoforms as therapeutic targets in nervous system disease states." Pharmacological Research 44(5):353-361, (2001); RN Frank: "Potential new medical therapies for diabetic retinopathy: protein kinase C inhibitors. Am J Ophthalmol 133:693-698 (2002); M Meier and GL King: "Protein kinase C activation and its pharmacological inhibition in vascular disease." Vascular Medicine 5:173-185 (2000)).

NFκB

Nuclear Factor κ B (NFκB) is a family of transcription factors found in a wide variety of mammalian cells. The mature molecule is a homo- or heterodimer, made from one or two of the following 5 gene products (RelA (p65), p50, RelB, c-Rel and p52) - the most common is a dimer of RelA and p50. Under non-activated conditions, NFκB is localized in the cytosol by association with an inhibitory protein IκBα. Upstream signaling involves an IκB kinase, and phosphorylation of the bound IκBα results in it's release from NFκB, allowing the later to translocate to the nucleus, and activate specific gene transcription. The phosphorylated IκBα is degraded by the ubiquitin – proteosome pathway.

NFκB is widely recognized as a key regulatory molecule associated with inflammation. Thus, it plays a key role in both acute and chronic inflammatory diseases (AB Lentsch and PA Ward: "Activation and regulation of NFκB during acute inflammation." Clin Chem Lab Med 37(3):205-208 (1999)). It also plays a role in certain aspects of other diseases, such as cancer metastasis (VB Andela, AH Gordon, G Zotalis, RN Rosier, JJ Goater, GD Lewis, EM Schwarz, JE Puzas and RJ O'Keefe: "NFκB: A pivotal transcription factor in prostate cancer metastasis to bone." Clinical Orthopaedics and Related Research 415S:S75-S85 (2003)). This transcription factor is involved in the development of the diabetic syndrome (E. Ho and TM Bray: "Antioxidants, NFκB activation and diabetogenesis." Proceedings of the Society for Experimental Biology and Medicine 222:205-213 (1999)) and in immune development and regulation (J Moscat, MT Diaz-

Meco and P Rennert: "NFκB activation by proptein kinase C isoforms and B-cell function." EMBO Reports 4:31-36 (2003)). Finally, NFκB is associated with control of apoptosis and in growth and differentiation. Indeed, PIF (proteolysis inducing factor, which is released by tumors and is involved in cancer – induced lean mass losses) is thought to be a regulator of enbryonic development, and triggers a signaling cascade ultimately through NFκB (F. Delfino and WH Walker: "Hormonal regulation of the NFκB signaling pathway." Molecular and Cellular Endocrinology 157:1-9 (1999); TM Watchorn, I Waddell, N Dowidar and JA Ross: "Proteolysis-inducing factor regulates hepatic gene expression via the transcription factor NFκB and STST3." FASEB J 15:562-564 (2001)).

It is also well known that EPA exerts it's beneficial effects on cachexia via inhibition of the signaling resulting from activation of PLA, in particular the release of arachidonic acid (AA). This prevents the subsequent upregulation and activation of the ubiquitin-proteosome pathway by removing the initial signaling event. HMB, while not preventing the activation of PLA or the release of AA, does prevent the upregulation of protein kinase C, preventing all subsequent activation in the signaling pathway, also ultimately preventing the activation of the ubiquitin-proteosome system.

It has now been surprisingly and unexpectedly discovered that HMB alone can reduce tumor growth rate and in combination with sub-optimal dose levels of EPA enhance the anticachectic effect. The combination of EPA and HMB preserve muscle mass by attenuating protein degradation through down regulation of the increased expression of key regulatory components of the ubiquitin-proteasome proteolytic pathway.

The term "HMB" refers to the compound having the foregoing chemical formula, in both its free acid and salt forms, metabolites and derivatives thereof. While any suitable form of HMB can be used within the context of the present invention, preferably, HMB is selected from the group consisting of a free acid, a salt, an ester, and a lactone; more preferably, HMB is a salt.

While any pharmaceutically suitable salt of HMB can be used within the context of the present invention, preferably, the HMB salt is water-soluble or becomes water-soluble in the stomach or intestines of a patient. More preferably, the HMB salt is selected from the group consisting of a sodium salt, a potassium salt, a magnesium salt, a chromium salt, and a calcium salt. Most preferably, the HMB salt is a calcium salt. However, other non-toxic salts, such as other alkali metal or alkaline earth metal salts, can be used.

Similarly, any pharmaceutically acceptable ester can be used in the context of the present invention. Desirably, the HMB ester is rapidly converted to HMB in its free acid form. Preferably, the HMB ester is a methyl ester or ethyl ester. HMB methyl ester and

HMB ethyl ester are rapidly converted to the free acid form of HMB.

Likewise, any pharmaceutically acceptable lactone can be used in the context of the present invention. Desirably, the HMB lactone is rapidly converted to HMB in its free acid form. Preferably, the HMB lactone is an isovalaryl lactone or a similar lactone. Such lactones are rapidly converted to the free acid form of HMB.

Methods for producing HMB and its derivatives are well known in the art. For example, HMB can be synthesized by oxidation of diacetone alcohol. One suitable procedure is described by Coffman et al., J. Am. Chem. Soc. 80: 2882-2887 (1958). As described therein, HMB is synthesized by an alkaline sodium hypochlorite oxidation of diacetone alcohol. The product is recovered in free acid form, which can be converted to the desired salt. For example, 3-hydroxy-3-methylbutyric acid (HMBA) can be synthesized from diacetone alcohol (4-hydroxy-4-methylpentan-2-one) via oxidation using cold, aqueous hypochlorite (bleach). After acidifying the reaction mixture using HCl, the HMBA product is recovered by extraction using ethyl acetate, and separating and retaining the organic layer from the extraction mixture. The ethyl acetate is removed by evaporation and the residue dissolved in ethanol. After addition of Ca(OH)2 and cooling, crystalline CaHMB can be recovered by filtration, the crystals washed with ethanol and then dried. Alternatively, the calcium salt of HMB is commercially available from TSI in Salt Lake City, Utah.

Nutritional support in the cancer patient can be categorized as (i) supportive, in which nutrition support is instituted to prevent nutrition deterioration in the adequately nourished patient or to rehabilitate the depleted patient before definitive therapy; (ii) adjunctive, in which nutrition support plays an integral role in the therapeutic plan; and (iii) definitive, in which aggressive nutrition support is required for the patient's existence. The routes for providing nutrition support include an oral diet, tube feeding and peripheral or total parenteral nutrition. The preferred embodiment for nutritional methods and compositions of the invention is by the oral route.

An alternate to oral feeding is tube feeding by means of nasogastric, nasoduodenal, esophagostomy, gastrostomy, or jejunostomy tubes.

The beneficial effects that HMB has on the lean body mass of a patient can be achieved in a number of ways. If desired, the HMB may be administered alone, without a carrier. The HMB may simply be dissolved in water and consumed by the patient. Alternatively, the HMB may be sprinkled on food, dissolved in coffee, etc. The total daily dose for the patient will vary widely, but typically a patient will benefit from consuming at least 2 gm/day of HMB. Alternatively, from 20 to 40 mg/kg/day.

In a further embodiment, the HMB may be incorporated into pills, capsules, rapidly dissolved tablets, lozenges, etc. The active dose can vary widely, but will typically range from 250 mg to 1gm/dose with the patient consuming from 2 to 8 doses/day to achieve the target of 2gm/day minimum. Methods for preparing such dosage forms are well known in the art. The reader's attention is directed to the most recent edition of Remingtons Pharmaceutical Sciences for guidance on how to prepare such dosage forms.

While the HMB may be administered as a single entity, it will typically be incorporated into food products and consumed by the patient during their meals or snack. If desired, the patient may simply modify the recipe of foods they normally consume by sprinkling on food, dissolving in coffee, etc.

In a further embodiment, the HMB will be incorporated into beverages, bars, cookies, etc. that have been specifically designed to enhance the palatability of the HMB and increase the selection of alternative forms, thereby enhancing patient/consumer acceptance.

Typically, the HMB will be incorporated into meal replacement beverages such as Ensure®, Boost®, Glucerna®, Pediasure®, Pedialyte®, etc. The HMB may also be incorporated into meal replacement bars such as PowerBars®, Glucerna® bars, Choice DM® bars, Ensure® bars, and Boost® bars, etc. Alternatively, the HMB maybe incorporated into juices, carbonated beverages, bottled water, etc. Additionally, the HMB may be incorporated into medical nutritonals such as ProSure®, Promote®, Jevity® and Advera® designed to support specific disease states such as cancer, HIV/AIDS, COPD arthritis, etc. Methods for producing any of such food products are well known to those skilled in the art. The following discussion is intended to illustrate such food products and their preparation.

Most meal replacement products (i.e., bars or liquids) provide calories from fat, carbohydrates, and protein. These products also typically contain vitamins and minerals, because they are intended to be suitable for use as the sole source of nutrition. While these meal replacement products may serve as the sole source of nutrition, they typically don't. Individuals consume these products to replace one or two meals a day, or to provide a healthy snack. The nutritional products of this invention should be construed to include any of these embodiments.

The amount of these nutritional ingredients can vary widely depending upon the targeted patient population (i.e., cancer, HIV/AIDS, arthritis, organoleptic considerations, cultural preferences, use, etc.). As a general nonlimiting guideline however, the meal replacement products of this invention will contain the following relative amounts of protein, fat, and carbohydrate (based upon the relative percentage of total calories): a protein component, providing from 5 to 80% of the total caloric content, a carbohydrate

component providing from 10 to 70% of the total caloric content, and a lipid component providing from 5 to 50% of the total caloric content.

The meal replacements will contain suitable carbohydrates, lipids and proteins as is known to those skilled in the art of making nutritional formulas. Suitable carbohydrates include, but are not limited to, hydrolyzed, intact, naturally and/or chemically modified starches sourced from corn, tapioca, rice or potato in waxy or non waxy forms; and sugars such as glucose, fructose, lactose, sucrose, maltose, high fructose corn syrup, corn syrup solids, fructooligosaccharides, and mixtures thereof.

Suitable lipids include, but are not limited to, coconut oil, soy oil, corn oil, olive oil, safflower oil, high oleic safflower oil, MCT oil (medium chain triglycerides), sunflower oil, high oleic sunflower oil, palm oil, palm olein, canola oil, cottonseed oil, fish oil, palm kernel oil, menhaden oil, soybean oil, lecithin, lipid sources of arachidonic acid and docosahexaneoic acid, and mixtures thereof. Lipid sources of arachidonic acid and docosahexaneoic acid include, but are not limited to, marine oil, egg yolk oil, and fungal or algal oil.

Numerous commercial sources for these fats are readily available and known to one practicing the art. For example, soy and canola oils are available from Archer Daniels Midland of Decatur, Illinois. Corn, coconut, palm and palm kernel oils are available from Premier Edible Oils Corporation of Portland, Organ. Fractionated coconut oil is available from Henkel Corporation of LaGrange, Illinois. High oleic safflower and high oleic sunflower oils are available from SVO Specialty Products of Eastlake, Ohio. Marine oil is available from Mochida International of Tokyo, Japan. Olive oil is available from Anglia Oils of North Humberside, United Kingdom. Sunflower and cottonseed oils are available from Cargil of Minneapolis, Minnesota. Safflower oil is available from California Oils Corporation of Richmond, California.

In addition to these food grade oils, structured lipids may be incorporated into the food product if desired. Structured lipids are known in the art. A concise description of structured lipids can be found in INFORM, Vol.. 8, No. 10, page 1004; entitled Structured lipids allow fat tailoring (October 1997). Also see United States Patent No. 4,871,768. Structured lipids are predominantly triacylglycerols containing mixtures of medium and long chain fatty acids on the same glycerol nucleus. Structured lipids and their use in enteral formula are also described in United States Patent Nos. 6,194,379 and 6,160,007.

Optionally, ω–3 fatty acids may comprise approximately 30% of the oil blend, preferably the ω–3 fatty acids largely consist of eicosapentaenoic acid and docosahexaenoic acid. Dietary oils used in the preparation of the nutritional composition generally contain ω–3 fatty acids in the triglyceride form and include, but are not limited to

canola, medium chain triglycerides, fish, soybean, soy lecithin, corn, safflower, sunflower, high-oleic sunflower, high-oleic safflower, olive, borage, black currant, evening primrose and flaxseed oil. Optionally, the weight ratio of ω–6 fatty acids to ω–3 fatty acids in the lipid blend according to the invention is about 0.1 to 3.0. The daily delivery of ω–3 fatty acids should be at least 450 mg and may vary depending on body weight, sex, age and medical condition of the individual. As mentioned, higher levels are desired for adult human consumption: for example, from about 0.5 to 50 gm daily, more preferably from about 2.5 to 5 gm daily.

An unexpected advantage to combining ω–3 fatty acids and HMB is the improvement in taste of the meal replacement. The typical sources of ω–3 fatty acids are fish and algae oils. Each source brings objectionable flavors to the meal replacement product. The Inventors discovered that by adding HMB, the same or better clinical results related to the prevention of involuntary weight loss can be obtained even when using sub-optimal or lower levels of ω–3 fatty acids in the product. Consequently, the Inventor's have discovered that there is an inverse relationship between the levels of ω–3 fatty acids and HMB. For example, if an effective does of ω–3 fatty acids is 3gm delivered in 2 cans of a meal replacement, the same clinical results would be seen in product formulated to contain 2gm of ω–3 fatty acids and 1gm of HMB delivered in 2 cans or in product formulated to contain 1gm of ω–3 fatty acids and 2gm of HMB delivered in 2 cans. The product formulated to contain only 1gm of ω–3 fatty acids will taste much better than the product formulated with 2 or 3 gm of ω–3 fatty acids while achieving the same clinical effectiveness. Further, since ω–3 fatty acids are known inhibitors of AA, a mediator of inflammation, a product containing ω–3 fatty acids and HMB could have broader benefits than those containing either of the ingredients alone.

Suitable protein sources include, but not limited to, milk, whey and whey fractions, soy, rice, meat (e.g., beef), animal and vegetable (e.g., pea, potato), egg (egg albumin), gelatin and fish. Suitable intact protein sources include, but are not limited to, soy based, milk based, casein protein, whey protein, rice protein, beef collagen, pea protein, potato protein, and mixtures thereof.

Optionally, the intact protein source is enriched in large neutral amino acids (LNAA) comprising valine, isoleucine, leucine, threonine, tyrosine and phenylalanine. Typically, about 40% of casein, whey and soy protein sources are large neutral amino acids. For example, caseinate contains about 38 wt/wt% LNAA , whey protein concentrate contains about 39 wt/wt% LNAA and soy protein isolate contains about 34 wt/wt% LNAA. Typically, the meal replacement is formulated with a protein source that will deliver about 1 to 25 gm of LNAA per day, preferably from about 1 to 20 gm of LNAA

per day, more preferably from about 4 to 20 gm of LNAA per day. As an example, a meal replacement consumed 3 times a day that contains a protein comprising 4.8 gm LNAA will deliver 14.4 gm LNAA per day.

The meal replacements preferably also contain vitamins and minerals in an amount designed to supply or supplement the daily nutritional requirements of the person receiving the formula. Those skilled in the art recognize that nutritional formulas often include overages of certain vitamins and minerals to ensure that they meet targeted level over the shelf life of the product. These same individuals also recognize that certain micro ingredients may have potential benefits for people depending upon any underlying illness or disease that the patient is afflicted with. For example, cancer patients benefit from such antioxidants as beta-carotene, vitamin E, vitamin C and selenium. The food products preferably include, but are not limited to, the following vitamins and minerals: calcium, phosphorus, sodium, chloride, magnesium, manganese, iron, copper, zinc, selenium, iodine, chromium, molybdenum, conditionally essential nutrients m-inositol, carnitine and taurine, and Vitamins A, C, D, E, K and the B complex, and mixtures thereof.

The conditionally essential nutrient carnitine is a naturally occurring amino acid formed from methionine and lysine. Its major metabolic role is associated with the transport of long-chain fatty acids across the mitochondrial membranes, thus stimulating the oxidation of these fuel substances for metabolic energy. Carnitine supplementation is an important metabolic tool in conditions such as diseases of the liver and kidney, and major chronic illnesses or extensive injuries complicated by malnutrition. Optionally, the meal replacements may be supplemented with carnitine at levels sufficient to supply up to 4gm/day of carnitine.

The meal replacements also may contain fiber and stabilizers. Suitable sources of fiber/and or stabilizers include, but are not limited to, xanthan gum, guar gum, gum arabic, gum ghatti, gum karaya, gum tracacanth, agar, furcellaran, gellan gum, locust bean gum, pectin, low and high methoxy pectin, oat and barley glucans, carrageenans, psyllium, gelatin, microcrystalline cellulose, CMC (sodium carboxymethylcellulose), methylcellulose hydroxypropyl methyl cellulose, hydroxypropyl cellulose, DATEM (diacetyl tartaric acid esters of mono- and diglycerides), dextran, carrageenans, FOS (fructooligosaccharides), and mixtures thereof. Numerous commercial sources of soluble dietary fibers are available. For example, gum arabic, hydrolyzed carboxymethylcellulose, guar gum, pectin and the low and high methoxy pectins are available from TIC Gums, Inc. of Belcamp, Maryland. The oat and barley glucans are available from Mountain Lake Specialty Ingredients, Inc. of Omaha, Nebraska. Psyllium is available from the Meer Corporation of North Bergen, New Jersey while the carrageenan is available from FMC Corporation of Philadelphia, Pennsylvania.

The fiber incorporated may also be an insoluble dietary fiber representative examples of which include oat hull fiber, pea hull fiber, soy hull fiber, soy cotyledon fiber, sugar beet fiber, cellulose and corn bran. Numerous sources for the insoluble dietary fibers are also available. For example, the corn bran is available from Quaker Oats of Chicago, Illinois; oat hull fiber from Canadian Harvest of Cambridge, Minnesota; pea hull fiber from Woodstone Foods of Winnipeg, Canada; soy hull fiber and oat hull fiber from The Fibrad Group of LaVale, Maryland; soy cotyledon fiber from Protein Technologies International of St. Louis, Missouri; sugar beet fiber from Delta Fiber Foods of Minneapolis, Minnesota and cellulose from the James River Corp. of Saddle Brook, New Jersey.

A more detailed discussion of examples of fibers and their incorporation into food products may be found in United States Patent No. 5,085,883 issued to Garleb et al.

The quantity of fiber utilized in the formulas can vary. The particular type of fiber that is utilized is not critical. Any fiber suitable for human consumption and that is stable in the matrix of a food product may be utilized.

In addition to fiber, the meal replacements may also contain oligosaccharides such as fructooligosaccharides (FOS) or glucooligosaccharides (GOS). Oligosaccharides are rapidly and extensively fermented to short chain fatty acids by anaerobic microorganisms that inhabit the large bowel. These oligosaccharides are preferential energy sources for most Bifidobacterium species, but are not utilized by potentially pathogenic organisms such as Clostridium perfingens, C. difficile, or Eschericia coli.

Typically, the FOS comprises from 0 to 5gm/serving of the meal replacement, preferably from 1 to 5gm/serving, more preferably from 2 to 4gm/serving of the meal replacement.

The meal replacements may also contain a flavor to enhance its palatability. Artificial sweeteners may be added to complement the flavor and mask salty taste. Useful artificial sweeteners include saccharin, nutrasweet, sucralose, acesulfane-K (ace-K), etc..

Meal replacements can be manufactured using techniques well known to those skilled in the art. Various processing techniques exist. Typically these techniques include formation of a slurry from one or more solutions, which may contain water and one or more of the following: carbohydrates, proteins, lipids, stabilizers, vitamins and minerals. The HMB is typically added to the carbohydrate slurry prior to the other minerals. The slurry is emulsified, homogenized and cooled. Various other solutions may be added to the slurry before processing, after processing or at both times. The processed formula is then sterilized and may be diluted to be dried to a powder, utilized on a ready-to-feed basis or packaged in a concentrated liquid form. When the resulting formula is meant to

be a ready-to-feed liquid or concentrated liquid, an appropriate amount of water would be added before sterilization.

Solid compositions such as bars, cookies, etc. may also be manufactured utilizing techniques known to those skilled in the art. For example, they may be manufactured using cold extrusion technology as is known in the art. To prepare such compositions, typically all of the powdered components will be dry blended together. Such constituents typically include the proteins, vitamin premixes, certain carbohydrates, etc. The fat-soluble components are then blended together and mixed with the powdered premix above. Finally any liquid components are then mixed into the composition, forming a plastic like composition or dough.

The process above is intended to give a plastic mass that can then be shaped, without further physical or chemical changes occurring, by the procedure known as cold forming or extrusion. In this process, the plastic mass is forced at relatively low pressure through a die, which confers the desired shape. The resultant exudate is then cut off at an appropriate position to give products of the desired weight. If desired the solid product is then coated, to enhance palatability, and packaged for distribution. Typically the package will provide directions for use by the end consumer (i.e., to be consumed by a cancer patient, to help prevent lean muscle loss, etc.)

The solid compositions of the instant invention may also be manufactured through a baked application or heated extrusion to produce cereals, cookies, and crackers. One knowledgeable in the arts would be able to select one of the many manufacturing processes available to produce the desired final product.

As noted above, the HMB may also be incorporated into juices, non-carbonated beverages, carbonated beverages, electrolyte solutions, flavored waters (hereinafter collectively "beverage"), etc. The HMB will typically comprise from 0.5 to 2 gm/serving of the beverages. Methods for producing such beverages are well known in the art. The reader's attention is directed to United States Patent No.'s 6,176,980 and 5,792,502, the contents of each which are hereby incorporated by reference. For example, all of the ingredients, including the HMB are dissolved in an appropriate volume of water. Flavors, colors, vitamins, etc. are then optionally added. The mixture is then pasteurized, packaged and stored until shipment.

Any disease with which wasting or inflammation is associated such as cardiovascular, peripheral microvascular, central nervous system, oncology, immune and infectious disease states can be treated in accordance with the present methods. Preferably, the disease is selected from the group consisting of cancer, cachexia, age-associated wasting, wasting associated with long-term hospital stay, HIV/AIDS, arthritis,

trauma, liver disease, Crohn's disease or other inflammatory bowel diseases (IBD), renal insufficiency and COPD (chronic obstructive pulmonary disease). More preferably, the disease is cachexia.

The present invention provides, in another embodiment, a method for the treatment of the disease-associated wasting of a patient, such as a mammal, preferably a human. The method comprises administering to the patient the above-described composition, which comprises HMB in amounts sufficient to treat the disease-associated wasting, wherein, upon administration of the composition to the patient, the disease-associated wasting is treated.

The amount of HMB that is sufficient to treat disease-associated wasting in a given patient can be determined in accordance with methods well known in the art. When treating the disease-associated wasting of a patient, desirably, the composition comprising HMB is administered to a patient suffering from disease-associated wasting in such an amount, in such a manner, and over such a period of time that the patient's lean tissue mass will increase without a concomitant decrease in the patient's fat mass. An example, within the context of treating the cancer cachexia associated wasting of a human, when the composition is orally administered about twice a day for a minimum of two weeks; the dose is sufficient to provide at least 2gm HMB/day.

The present invention provides, in another embodiment, a method for reducing tumor growth rate in a patient, such as a mammal, preferably a human. The method comprises administering to the patient the above-described composition, which comprises HMB in amounts sufficient to reduce tumor growth rate, wherein, upon administration of the composition to the patient, the tumor growth rate is reduced.

The amount of HMB that is sufficient to attenuate tumor growth in a given patient can be determined in accordance with methods well known in the art. When treating tumor growth in a patient, desirably, the composition comprising HMB is administered to a patient suffering from tumor growth in such an amount, in such a manner, and over such a period of time that the patient's tumor growth rate will decrease. An example, within the context of treating the tumor growth in an adult human, when the composition is orally administered about twice a day for a minimum of two weeks; the dose is sufficient to provide at least about 2 gm HMB/day.

The present invention provides, in another embodiment, a method for down regulating the expression and/or activity of protein kinase C. Examples I – IV show that both EPA and HMB attenuated PIF-induced activation of protein kinase C (PKC) and the subsequent degradation of IκBα and nuclear accumulation of nuclear factor-κB (NF-κB).

The present invention provides, in another embodiment, a method for down regulating the expression and/or activity of nuclear factor kappa-B. Examples I – IV show that both EPA and HMB attenuated PIF-induced activation of protein kinase C (PKC) and the subsequent degradation of IκBα and nuclear accumulation of nuclear factor-κB (NF-κB).

The present invention provides, in another embodiment, a method for down regulating the expression and/or activity of ubiquitin-conjugating enzymes. Examples I – IV show that this was accompanied by a reduction in the expression of $E2_{14k}$ ubiquitin-conjugating enzyme. The combination of EPA and HMB was at least as effective or more effective than either treatment alone. These results show that both EPA and HMB preserve muscle mass by attenuating protein degradation through down regulation of the increased expression of key regulatory components of the ubiquitin-proteasome proteolytic pathway.

The present invention provides, in another embodiment, a method for down regulating the expression and/or activity of components of 26S proteasome. Examples I – IV show that proteasome activity, determined by the 'chymotrypsin-like' enzyme activity, was attenuated by HMB. Protein expression of the 20S α or β-subunits was reduced by at least 50%, as were the ATPase subunits MSS1 and p42 of the 19S proteasome regulatory subunit.

Example I

Prevention of Weight Loss and Attenuation of Protein Degradation in Animals with Cancer Cachexia

This study evaluates the effect of HMB, in comparison with EPA or combination, on weight loss induced by the MAC16 tumor and the mechanisms involved. Weight loss induced by the MAC16 tumor is primarily induced by PIF.

Pure strain male NMRI mice (average weight 25g) were obtained from our own inbred colony and were transplanted with fragments of the MAC16 tumor s.c. into the flank by means of a trochar, selecting from donor animals with established weight loss as described in Bibby, M.C. et al. Characterization of a transplantable adenocarcinoma of the mouse colon producing cachexia in recipient animals. J. Natl. Cancer Inst., 78: 539-546, 1987. Transplanted animals were fed a rat and mouse breeding diet (Special Diet Services, Witham, United Kingdom) and water *ad libitum*, and weight loss was evident 10-12 days after tumor implantation. Animals just prior to the development of weight loss were randomized to receive daily either EPA (in olive oil), HMB (in PBS) or the combination as described in the figure legends administered p.o. by gavage, while control

animals received either olive oil or PBS. EPA (98% as free acid) was purchased from Biomol Research Laboratories Inc., PA, USA. HMB (as the calcium salt) was obtained from Abbott Laboratories, Columbus, Ohio, USA. All groups contained a minimum of 6 mice. Tumor volume, body weight and food and water intake were monitored daily. Animals were terminated by cervical dislocation when the body weight loss reached 25%, and all studies were conducted according to the UKCCR Guidelines for the care and use of laboratory animals. The soleus muscles were quickly dissected out, together with intact tendons, and maintained in isotonic ice-cold saline before determination of protein degradation.

Freshly dissected soleus muscles were fixed via the tendons to aluminium wire supports, under tension, at approximately resting length to prevent muscle shortening and preincubated for 45 min in 3ml of oxygenated (95% oxygen : 5% carbon dioxide) Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 5mM glucose and 0.5mM cycloheximide. Protein degradation was determined by the release of tyrosine over a 2h period as described in Waalkes, T.P. et al. A fluorimetric method for the estimation of tyrosine in plasma and tissues. J. Lab. Clin. Med., 50: 733-736, 1957.

Functional proteasome activity was determined by measuring the 'chymotrypsin-like' enzyme activity, the predominant proteolytic activity of the β-subunits of the proteasome according to the method of Orino, E. et al. ATP-dependent reversible association of proteasomes with multiple protein components to form 26S complexes that degrade ubiquitinated proteins in human HL-60 cells. FEBS Lett., 284: 206-210, 1991. Muscles were rinsed with ice-cold PBS, minced and sonicated in 20mM Tris. HCl, pH 7.5, 2mM ATP, 5mM $MgCl_2$ and 1mM DTT. The sonicate was then centrifuged for 10 min at 18,000g, at 4°C and the supernatant was used to determine 'chymotrypsin-like' enzyme activity by the release of aminomethyl coumarin (AMC) from the fluorogenic substrate succinyl – LLVY - AMC. Activity was measured in the absence and presence of the specific proteasome inhibitor lactacystin (10μM). Only lactacystin suppressible activity was considered to be proteasome specific.

For Western blotting samples of soleus muscle cytosolic protein (2 to 5μg), obtained from the above assay, were resolved on 10% SDS-PAGE and transferred to 0.45μm nitrocellulose membrane (Hybond™, Amersham Life Science Products, Bucks, United Kingdom), which had been blocked with 5% Marvel in PBS. The primary antibodies for MSS1 and p42 were used at a dilution of 1:5000, for 20S proteasome α-subunits at 1:1500 and for β-subunits at 1:1000, while the antibody for $E2_{14k}$ was used at a dilution of 1:500. The secondary antibodies were used at a dilution of 1:2000. Mouse monoclonal antibodies to 20S proteasome subunits α 1, 2, 3, 5, 6 and 7 (clone MCP 231),

20S proteasome subunit β3 (HC10), 19S regulator ATPase subunit Rpt 1(S7, Mss1; clone MSS1-104) and 19S regulator ATPase subunit Rpt 4 (S106, p42; clone p42-23) were purchased from Affiniti Research Products, Exeter, United Kingdom. Rabbit polyclonal antisera to ubiquitin-conjugating enzyme E2 (anti-UBC2 antibody) was a gift from Dr. Simon Wing, McGill University, Montreal, Quebec, Canada. Peroxidase-conjugated goat anti-rabbit and rabbit anti-mouse secondary antibodies were from Dako Ltd., Cambridge, United Kingdom. Incubation was carried out for 2h at room temperature, and developed by chemiluminescence (ECL; Amersham).

A dose-response relationship of HMB on weight loss in mice bearing the MAC16 tumor is shown in Figure 2. Doses of HMB greater than 0.125g/kg caused a significant reduction in weight loss (Figure 2A). Differences from the control group are indicated as a, $p<0.05$; b, $p<0.01$ and c, $p<0.005$. Attenuation of weight loss was not accompanied by an alteration in food and water intake. A dose level of 0.25g/kg was chosen for all subsequent experiments. The effect of HMB, EPA and the combination of HMB and EPA on weight loss in MAC16 cachectic tumour-bearing mice is shown in Figure 3. Differences from the control group are indicated as a, $p<0.05$; b, $p<0.01$ or c, $p<0.005$. A suboptimal dose of EPA was chosen to investigate interactions with HMB. All treatments caused a significant increase in soleus muscle weight (Figure 4A), and a significant reduction in tyrosine release (Figure 4B), indicating a reduction in total protein degradation. Differences from the PBS control group are indicated as a, $p<0.05$, b, $p<0.01$ or c, $p<0.005$. At the doses chosen, HMB was as effective as EPA.

Proteasome expression has been shown to be elevated in gastrocnemius muscles of mice bearing the MAC16 tumor and this increased gene expression has been shown to be attenuated by EPA. The results in Figure 5 show that functional proteasome activity, as determined by 'chymotrypsin-like' enzyme activity, was attenuated by HMB to the same extent as EPA at the doses chosen, and that the combination of HMB and EPA did not produce a further depression in activity. Differences from control are indicated as c, $p<0.005$. Protein expression of proteasome subunits was analysed by Western blotting of supernatants from sonicated muscle tissues. Expression of 20S proteasome α-subunits, the structural units of the proteasome was attenuated by both HMB and EPA, and there was some indication of a further decrease of band 2 for the combination(Figure 6A). Differences from control are shown as c, $p<0.001$, while differences from HMB are shown as e, $p<0.01$. Expression of the 20S proteasome β-subunits, the catalytic subunits of the proteasome, were also attenuated by HMB and EPA, but the combination was more effective than either agent alone (Figure 6B). Differences from control are shown as c, $p<0.001$.

Expression of MSS1, an ATPase subunit of the 19S proteasome regulatory complex is shown in Figure 7A. Both HMB and EPA attenuated MSS1 expression, but the combination did not appear to produce a further reduction. Similar results were obtained with p42, another ATPase subunit of the 19S regulator, that promotes ATP dependent association of the 20S proteasome with the 19S regulator to form the 26S proteasome (Figure 7B). Differences from control are shown as c, $p<0.001$. Again both HMB and EPA appeared to be equally effective, while the combination did appear to reduce p42 expression further. Expression of the ubiquitin-conjugating enzyme, $E2_{14k}$, was also reduced by both HMB and EPA, while the combination caused a further reduction in expression (Figure 8). Differences from control are shown as b, $p<0.01$ and c, $p<0.001$, while differences from HMB alone are shown as d, $p<0.05$ and f, $p<0.001$. These results confirm HMB to be as effective as EPA in attenuating loss of muscle mass, protein degradation and down-regulation of the ubiquitin-proteasome proteolytic pathway, and this mechanism appears to be responsible for the preservation of muscle mass in cachectic mice bearing the MAC16 tumor.

This study has shown that HMB is effective in attenuating the development of cachexia or involuntary weight loss in mice bearing the MAC16 tumor and produced a reduction in protein degradation in skeletal muscle by down regulating the increased expression of the ubiquitin-proteasome pathway. Thus HMB is as effective as EPA in reducing protein expression of the 20S proteasome α and β subunits, as well as two subunits of the 19S regulator MSS1 and p42, expression of $E2_{14k}$ and proteasome proteolytic activity.

Example II
Attenuation of Tumor Growth in Animals

The animal study described in Example I above also evaluated the effect of HMB on tumor growth rate in MAC16 cachectic tumor-bearing mice. The experiment was conducted as described in Example I.

A dose-response relationship of HMB alone on tumor growth rate in mice bearing the MAC16 tumor is shown in Figure 2B. Differences from the control group are indicated as a, $p<0.05$; b, $p<0.01$ and c, $p<0.005$. Doses of HMB greater than 0.125g/kg caused a significant reduction in tumor growth rate. Attenuation of tumor growth was not accompanied by an alteration in food and water intake.

Example III
Attenuation of Protein Degradation in Murine Myotubes

This study examines the effect of HMB on PIF-induced protein degradation and signalling pathways in murine myotubes to determine the mechanism of the attenuation of the increased expression of the ubiquitin-proteasome proteolytic pathway.

C_2C_{12} myotubes were routinely passaged in DMEM supplemented with 10% FCS, glutamine and 1% penicillin-streptomycin under an atmosphere of 10% CO_2 in air at 37□C. Myotubes were formed by allowing confluent cultures to differentiate in DMEM containing 2% HS, with medium changes every 2 days.

PIF was purified from solid MAC16 tumors (Todorov, P. et al. Characterization of a cancer cachectic factor. Nature, 379: 739-742, 1996.) excised from mice with a weight loss of 20 to 25%. Tumors were homogenised in 10mM Tris-HCl, pH 8.0, containing 0.5mM phenylmethylsulfonyl fluoride, 0.5mM EGTA and 1mM dithiothreitol at a concentration of 5ml/g tumor. Solid ammonium sulfate was added to 40% w/v and the supernatant, after removal of the ammonium sulfate, was subjected to affinity chromatography using anti-PIF monoclonal antibody coupled to a solid matrix as described in Todorov, P. et al Induction of muscle protein degradation and weight loss by a tumor product. Cancer Res., 56: 1256-1261, 1996. The immunogenic fractions were concentrated and used for further studies.

Myotubes in six-well multidishes were labeled with L-[2, 6-^{3}H] phenylalanine (0.67mCi/mmole) for 24h in 2ml DMEM containing 2% HS. They were then washed three times in PBS followed by a 2h incubation at 37°C in DMEM without phenol red until no more radioactivity appeared in the supernatant. These myotubes were then further incubated for 24h in the presence of PIF, with and without EPA or HMB, in fresh DMEM without phenol red, to prevent quenching of counts, and in the presence of 2mM cold phenylalanine to prevent reincorporation of radioactivity. The amount of radioactivity released into the medium was expressed as a percentage of control cultures not exposed to PIF to determine total protein degradation.

For measurement of arachidonic acid release, myotubes in six-well multi dishes containing 2ml DMEM with 2% HS were labeled for 24h with 10μM arachidonic acid (containing 1μCi of [^{3}H] arachidonate/ml) (Smith, H. et al. Effect of a cancer cachectic factor on protein synthesis / degradation in murine C_2C_{12} myoblasts: Modulation by eicosapentaenoic acid. Cancer Res., 59: 5507-5513, 1999). Cells were then washed extensively with PBS to remove traces of unincorporated [^{3}H] arachidonate and either EPA or HMB was added 2h prior to PIF. After a further 24h 1ml of medium was removed to determine the radioactivity released.

The functional activity of the β subunits of the proteasome was determined as the 'chymotrypsin-like' enzyme activity obtained fluorimetrically according to the method of

Orino, E. et al. ATP-dependent reversible association of proteasomes with multiple protein components to form 26S complexes that degrade ubiquitinated proteins in human HL-60 cells. FEBS Lett., 284: 206-210, 1991. Myotubes were exposed to PIF for 24h with or without EPA or HMB added 2h prior to PIF and enzyme activity was determined in a supernatant fraction (Whitehouse, A.S. et al. Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-κB. Br. J. Cancer, 89: 1116-1122, 2003) by the release of aminomethyl coumarin (AMC) from succinyl-LLVY-AMC (0.1mM) in the presence or absence of the specific proteasome inhibitor lactacystin (10μM) (Fenteany, G. et al. Lactacystin, proteasome function and cell fate. J. Biol. Chem., 273: 8545-8548, 1998). Only lactacystin suppressible activity was considered to be proteasome specific. Activity was adjusted for the protein concentration of the sample, determined using the Bradford assay (Sigma Chemical Co., Dorset, United Kingdom) using bovine serum albumin as standard.

For Western blot analysis, cytosolic protein (2 to 5μg) obtained for the above assay were resolved on 10% SDS-PAGE and transferred to 0.45μm nitrocellulose membrane, which had been blocked with 5% Marvel in PBS, at 4°C overnight. The primary antibodies were used at a dilution of 1:100 (anti-actin and PKC_{α}); 1:500 (anti-ERK1 and 2); 1:1000 (anti-20S proteasome β-subunit and I-κBα); 1:1500 (anti-20S proteasome α-subunit) or 1:5000 (anti-p42), while the secondary antibodies were used at a dilution of 1:2000. Incubation was carried out for 2h at room temperature and development was by ECL. Loading was quantitated by actin concentration.

DNA binding proteins were extracted from myotubes by the method of Andrews, N.C. et al. A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. Nucleic Acids Res., 19: 2499, 1991, which utilizes hypotonic lysis followed by high salt extraction of nuclei. The EMSA (electrophoretic mobility shift assay) binding assay was carried out according to the manufacturer's instructions.

Since protein degradation and activation of the ubiquitin-proteasome proteolytic pathway in mice bearing the MAC16 tumor is thought to be mediated by PIF, mechanistic studies on the effect of HMB on protein degradation were carried out in murine myotubes treated with PIF. PIF-induced total protein breakdown with a typical bell-shaped dose-response curve, as previously reported by Gomes-Marcondes, et al Development of an in-vitro model system to investigate the mechanism of muscle protein catabolism induced by proteolysis-inducing factor. Br. J. Cancer, 86: 1628-1633, 2002 with a maximal effect at 4nM. The effect of EPA has been previously shown (Smith, H.J. et al. Effect of a cancer

cachectic factor on protein synthesis / degradation in murine C_2C_{12} myoblasts: Modulation by eicosapentaenoic acid. Cancer Res., 59: 5507-5513, 1999; Whitehouse, A.S. et al. Induction of protein catabolism in myotubes by 15(S)-hydroxyeicosatetraenoic acid through increased expression of the ubiquitin-proteasome pathway. Br. J. Cancer, 89: 737-745, 2003; and Whitehouse, A.S. et al. Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-κB. Br. J. Cancer, 89: 1116-1122, 2003) to be effective at 50μM, and the data in Figure 9A shows that at a concentration of 50μM both HMB and EPA were equally effective in attenuating PIF induced protein degradation. There was also some attenuation at 25μM HMB at low, but not at high concentrations of PIF. Differences from control in the absence of PIF are indicated as a, $p<0.005$, while differences form control with PIF (for groups with additions of HMV or EPA) are indicated as b, $p<0.01$ and c, $p<0.005$.

PIF-induced protein degradation has previously been shown to be due to an increased expression of the regulatory components of the ubiquitin-proteasome proteolytic pathway by Lorite, M.J., Smith, H.J., Arnold, J.A., Morris, A., Thompson, M.G. and Tisdale, M. J. Activation of ATP-ubiquitin-dependent proteolysis in skeletal muscle in vivo and murine myoblasts in vitro by a proteolysis-inducing factor (PIF). Br. J. Cancer, 85: 297-302, 2001 and Gomes-Marcondes, M.C.C., Smith, H.J., Cooper, J.C. and Tisdale, M.J. Development of an *in-vitro* model system to investigate the mechanism of muscle protein catabolism induced by proteolysis-inducing factor. Br. J. Cancer, 86: 1628-1633, 2002.

The functional activity of this pathway is measured by the 'chymotrypsin-like' enzyme activity, the predominant proteolytic activity of the β-subunits of the proteasome. PIF induced an increase in 'chymotrypsin-like' enzyme activity, which was maximal at 4.2nM. The effect of PIF was completely attenuated by 50μM EPA and both 25 and 50μM HMB. (Figure 9B, differences from control are shown as a, $p<0.001$, while differences in the presence of EPA or HMB are shown as b, $p<0.001$). A similar effect was observed on expression of proteasome 20S α subunits, β subunits and p42, an ATPase subunit of the 19S regulator that promotes ATP-dependent association of the 20S proteasome with the 19S regulator to form the 26S proteasome (Fig. 10). In all cases expression was increased by PIF at 4.2 and 10nM and this was attenuated by EPA and HMB at 50μM, but not at 25μM. These results confirm that HMB attenuates protein degradation through an effect on PIF induction of the ubiquitin-proteasome pathway.

Example IV

Effect on Activity of Mediator of Signaling in Inflammation and Proteolysis

The *in vitro* study described in Example III above also evaluated the effect of HMB on molecules that are key mediators in the pathway of inflammation. This experiment was conducted as described in Example III.

Activation of PKC has been shown to activate extracellular signal-regulated kinase (ERK) cascade of MAPK signalling pathways (Toker, A. Signalling through protein kinase C. Front. Biosci., 3: 1134-1147, 1998; Wolf, I and Seger, R. The mitogen-activated protein kinase signalling cascade: from bench to bedside. IMAJ., 4: 641-647). The activated ERKs, e.g., ERK1 (or p44 MAPK) and ERK2 (or p42 MAPK), are able to phosphorylate and consequently activate cytosolic phospholipase A2, the rate-limiting enzyme in pathways involving arachidonic acid release in inflammation. In addition, PIF has been shown to induce phosphorylation of p42 / 44 MAPK, while the total MAPK remained unchanged and to be involved in PIF-induced proteasome expression (Smith, H.J. et al. Signal transduction pathways involved in proteolysis-inducing factor induced proteasome expression in murine myotubes. Br. J. Cancer, 89: 1783-1788, 2003). The effect of EPA and HMB on this process is shown in Figure 12. PIF induced an increased phosphorylation of p42 / 44 that was maximal at 4.2nM and this effect was completely attenuated by both EPA and HMB at 50μM, but not HMB at 25μM. The ability of HMB to attenuate ERK 1/2 phosphorylation may be important in inhibition of PIF-induced proteasome expression by HMB.

Experiments using mutants of PKC as well as inhibitors of this enzyme show that this forms a central mediator of intracellular signalling by PIF. PKC is likely to be involved in phosphorylation (and degradation) of I-κBα leading to nuclear accumulation of NF-κB and increased gene transcription. PIF stimulates translocation of PKC_{α} from the cytoplasm to the plasma membrane (Fig. 11) resulting in activation with a maximum effect at 4.2nM PIF as with protein degradation (Fig. 9). This process was effectively attenuated by both EPA and HMB at 50μM; while HMB was less effective at 25μM (Fig. 11). This suggests that PIF-induced stimulation of PKC is attenuated by HMB through inhibition of PKC.

As previously discussed PIF induces degradation of I-κBα and stimulates nuclear accumulation of NF-κB and this process has been shown to be attenuated by 50μM EPA (Whitehouse, A.S. et al. Increased expression of the ubiquitin-proteasome pathway in murine myotubes by proteolysis-inducing factor (PIF) is associated with activation of the transcription factor NF-κB. Br. J. Cancer, 89: 1116-1122, 2003). The results in Figure 13A show HMB at 50μM to effectively attenuate I-κBα degradation in the presence of PIF in murine myotubes, and prevent nuclear accumulation of NF-κB (Figure 13C). Differences from 0nM PIF are shown as b, $p<0.01$ and c, $p<0.001$. Only partial inhibition

of binding of NF-κB to DNA was observed when HMB was used at a concentration of 25μM (Figure 13B). Differences from 0nM PIF b = p<0.01 and c=p<0.001. Differences between 50uM HMB and PIF treated against PIF alone at the same concentration e=p<0.01 and f=p<0.001. These results suggest that the overall effect of HMB is comparable to that of EPA in preventing movement of NF-κB into the nucleus with concomitant activation of gene expression.

Thus HMB appears to be an effective agent in the treatment of cytokine induced inflammation and muscle wasting in cancer cachexia. HMB appears to exert its effect by inhibition of PKC activity, and resultant stabilization of the cytoplasmic IκB / NF-κB complex. Since these molecules are key mediators in the pathway of inflammation, HMB appears to be an anti-inflammatory compound.

EXAMPLE V
Composition of a Nutritional Product to Prevent Involuntary Weight Loss

The specific list of materials for manufacturing the nutritional product of this Example is presented in Table 1. Of course, various changes in specific ingredients and quantities may be made without departing from the scope of the invention.

TABLE 1: LIST OF MATERIALS

INGREDIENT	AMOUNT (KG)
WATER	316
ULTRATRACE/TRACE MINERAL PREMIX	0.06
ZINC SULFATE	0.033
MANGANESE SULFATE	0.0082
SODIUM MOLYBDATE	0.00023
CHROMIUM CHLORIDE	0.00029
SODIUM SELENITE	0.000098
POTASSIUM CHLORIDE	0.072
SODIUM CITRATE	2.89
POTASSIUM IODIDE	0.00009
POTASSIUM CITRATE	1.5
CORN SYRUP	7.68
MALTODEXTRIN	53.6
MAGNESIUM PHOSPHATE DIBASIC	0.26
CALCIUM PHOSPHATE TRIBASIC	0.99
MAGNESIUM CHLORIDE	1.2
SUCROSE	11.9
FRUCTOOLIGOSACCHARIDE	5.9
MEDIUM CHAIN TRIGLYCERIDE	2.6
CANOLA OIL	1.5

INGREDIENT	AMOUNT (KG)
SOY OIL	0.87
57% VITAMIN A PALMITATE	0.007
VITAMIN DEK PREMIX	0.04
VITAMIN D	0.0000088
D-ALPHA-TOCOPHEROL ACETATE	0.036
PHYLLOQUINONE	0.00006
CARRAGEENAN	0.03
SOY LECITHIN	0.6
SODIUM CASEINATE	15.5
CALCIUM CASEINATE	4.2
CALCIUM HMB MONOHYDRATE	2.6
MILK PROTEIN ISOLATE	14
REFINED DEODORIZED SARDINE OIL	6.9
ASCORBIC ACID	0.12
45% POTASSIUM HYDROXIDE	0.13
TAURINE	0.12
WATER SOLUBLE VITAMIN PREMIX	0.11
NIACINAMIDE	0.017
CALCIUM PANTOTHENATE	0.01
THIAMINE CHLORIDE HYDROCHLORIDE	0.003
PYRIDOXINE HYDROCHLORIDE	0.003
RIBOFLAVIN	0.002
FOLIC ACID	0.0004
BIOTIN	0.00034
CYANOCOBALAMIN	0.000038
ASCORBYL PALMITATE	0.03
CHOLINE CHLORIDE	0.25
L-CARNITINE	0.0681
N&A MARSHMALLOW VANILLA	1.6
N&A DULCE DE LECHE	0.27

The liquid nutritional product of the present invention was manufactured by preparing three slurries which are blended together, combined with refined deodorized sardine oil, heat treated, standardized, packaged and sterilized. The process for manufacturing 454 kg (1,000 pounds) of the liquid nutritional product, using the List of Materials from Table 7, is described in detail below.

A carbohydrate/mineral slurry is prepared by first heating about 62.6 kg of water to a temperature in the range of about 71 °C to 77 °C with agitation. The HMB is added to the water and dissolved by agitaiting the resultant solution for at least five minutes. The required amount of potassium citrate and ultratrace/trace mineral premix is added to the water and dissolved by agitating the resultant solution for at least 10 minutes. The following minerals are then added, in the order listed, with high agitation: magnesium chloride, potassium chloride, sodium citrate, potassium iodide, magnesium phosphate and tricalcium phosphate. The slurry is allowd to mix under moderate agitation until completely dissolved or dispersed. The corn syrup, sucrose and maltodextrin are then added to the slurry with agitation. Add the required amount of FOS and allow to mix. The completed carbohydrate/mineral slurry is held with high agitation at a temperature in the

range of about 60 - 66 °C for not longer than 8 hours until it is blended with the other slurries.

An oil slurry is prepared by combining and heating the medium chain triglycerides (fractionated coconut oil), canola oil and soy oil to a temperature in the range of about 32 - 43 °C with agitation. The vitamin DEK premis is added and allowed to mix until completely dispersed. The required amounts of following ingredients are added: sly lecithin, vitamin A, ascorbyl plamitate, and vitamin E. The carrageen is added and allowed to mix until completely dispersed. The completed oil slurry is held under moderate agitation at a temperature in the range of about 32 - 43 °C for not longer than 8 hours until it is blended with the other slurries.

A protein slurry is prepared by first heating about 196.78 kg of water to a temperature in the range of about 60 - 63 °C with agitation. The calcium caseinate and sodium caseinate and milk protein isolate are blended into the slurry using a mixing apparatus. The completed protein slurry is held under agitation at a temperature in the range of about 54 - 60 °C for not longer than 2 hours before being blended with the other slurries.

The oil and the protein slurry are blended together with agitation and the resultant blended slurry is maintained at a temperature in the range of about 54 - 66 °C. After waiting for at least five minutes the carbohydrate/mineral slurry is added to the blended slurry from the preceding step with agitation and the resultant blended slurry is maintained at a temperature in the range of about 54 - 66 °C. The refined deodorized sardine oil is then added to the slurry with agitation. (In a most preferred method of manufacture the sardine oil would be slowly metered into the product as the blend passes through a conduit at a constant rate.) Preferably after at least 5 minutes the pH of the blended slurry is determined. If the pH of the blended slurry is below 6.55, it is adjusted with dilute potassium hydroxide to a pH of 6.5 to 6.8.

After waiting a period of not less than one minute nor greater than two hours the blended slurry is subjected to deaeration, Ultra-High-Temperature (UHT) treatment, and homogenization, as described as follows: use a positive pump for supplying the blended slurry for this procedure; heat the blended slurry to a temperature in the range of about 66 - 71 °C; deaerate the blended slurry to 25.4 - 38.1 cm of Hg; emulsify the blended slurry at 61-75 Atmospheres; heat the blended slurry to a temperature in the range of about 120 - 122 °C by passing it through a plate/coil heat exchanger with a hold time of approximately 10 seconds; UHT heat the blended slurry to a temperature in the range of about 144 - 147 °C with a hold time of approximately 5 seconds; reduce the temperature of the blended slurry to be in the range of about 120-122 °C by passing it through a flash cooler; reduce

the temperature of the blended slurry to be in the range of about 71 - 82 °C by passing it through a plate/coil heat exchanger; homogenize the blended slurry at about 265 to 266 Atmospheres; pass the blended slurry through a hold tube for at least 16 seconds at a temperature in the range of about 74 - 85 °C; and cool the blended slurry to a temperature in the range of about 1 - 70 °C by passing it through a large heat exchanger.

Store the blended slurry at a temperature in the range of about 1 - 7 °C, preferably with agitation.

Preferably at this time appropriate analytical testing for quality control is conducted. Based on the test results an appropriate amount of dilution water (10-38 °C) is added to the blended slurry with agitation.

A vitamin solution and flavor solution are prepared separately and then added to the blended slurry.

The vitamin solution is prepared by heating about 3.94 kg of water to a temperature in the range of about 43 - 66 °C with agitation, and thereafter adding the following ingredients, in the order listed: Ascorbic Acid, 45% Potassium Hydroxide, Taurine, Water Soluble Vitamin Premix, Choline Chloride, and L-Carnitine. The vitamin solution is then added to the blended slurry with agitation.

The flavor solution is prepared by adding the marshmallow and dulce de leche flavor to about 7.94 kg of water with agitation. A nutritional product according to the present invention has been manufactured using an artificial marshmallow flavor distributed by Firmenich Inc., Princeton, New Jersey, U.S.A. and a natural & artificial dulce de leche flavor distributed by Firmenich Inc. The flavor solution is then added to the blended slurry with agitation.

If necessary, diluted potassium hydroxide is added to the blended slurry such that the product will have a pH in the range of 6.4 to 7.0 after sterilization. The completed product is then placed in suitable containers and subjected to sterilization. Of course, if desired aseptic processing could be employed.

Example VI

Composition of a Nutritional Product to Control Glycemic Response

Table 2 presents a bill of materials for manufacturing 1,000 kg of a liquid nutritional product, which provides nutrients to a person but limits resulting insulin response. A detailed description of its manufacture follows.

Table 2: Bill of Materials for a Liquid Nutritional

Ingredient	Quantity per 1,000 kg

Water	QS
Maltodextrin	56 kg
Acidc casein	41.093 kg
Fructose	28 kg
High oleic safflower oil	27.2 kg
Maltitol syrup	16 kg
Maltitol	12.632 kg
Fibersol® 2(E)	8.421 kg
Caseinate	6.043 kg
Fructooligosaccharide	4.607 kg
Soy polysaccharide	4.3 kg
Canola oil	3.2 kg
Tricalcium phosphate	2.8 kg
Magnesium chloride	2.4 kg
Lecithin	1.6 kg
Sodium citrate	1.18 kg
Potassium citrate	1.146 kg
Sodium hydroxide	1.134 kg
Magnesium phosphate	1.028 kg
Calcium HMB monohydrate	5.7 kg
m-inositol	914.5 gm
Vitamin C	584 gm
Potassium chloride	530 gm
Choline chloride	472.1 gm
45% Potassium hydroxide	402.5 gm
UTM/TM premix	369.3 gm
Potassium phosphate	333 gm
Carnitine	230.5 gm
Gellan gum	125 gm
Ttaurine	100.1 gm
Vitamin E	99 gm
Lutein Esters (5%)	92 gm
WSV premix	75.4 gm
Vitamin DEK premix	65.34 gm
30% Beta carotene	8.9 gm
Vitamin A	8.04 gm
Pyridoxine hydrochloride	3.7 gm
Chromium chloride	1.22 gm
Folic acid	0.64 gm
Potassium iodide	0.20 gm
Cyanocobalamin	0.013 gm

WSV premix(per g premix): 375 mg/g niacinamide, 242 mg/g calcium pantothenate, 8.4 gm/g folic acid, 62 mg/g thiamine chloride hydrochloride, 48.4 gm/g riboflavin, 59.6 mg/g pyridoxine hydrochloride, 165 mcg/g cyanocobalamin and 7305 mcg/g biotin

Vitamin DEK premix(per g premix): 8130 IU/g vitamin D_3, 838 IU/g vitamin E, 1.42 mg/g vitamin K_1

UTM/TM premix(per g premix): 45.6 mg/g zinc, 54 mg/g iron, 15.7 manganese, 6.39 mg/g copper, 222 mcg/g selenium, 301 mcg/g chromium and 480 mcg/g molybdenium

The diabetic liquid nutritional products of the present invention are manufactured by preparing four slurries that are blended together, heat treated, standardized, packaged and sterilized.

A carbohydrate/mineral slurry is prepared by first heating about 82 kg of water to a temperature of from about 65°C to about 71°C with agitation. With agitation, the required mount of calcium HMB is added and agitated for 5 minutes. The required amount of sodium citrate and gellen gum distributed by the Kelco, Division of Merck and Company Incorporated, San Diego, California, U.S.A. is added and agitated for 5 minutes. The required amount of the ultra trace mineral/trace mineral (UTM/TM) premix (distributed by Fortitech, Schnectady, New York) is added. The slurry is greenish yellow in color. Agitation is maintained until the minerals are completely dispersed. With agitation, the required amounts of the following minerals are then added: potassium citrate, potassium chloride, chromium chloride, magnesium chloride and potassium iodide. Next, the first maltodextrin distributed by Grain Processing Corporation, Muscataine, Iowa, U.S.A. and fructose are added to slurry under high agitation, and are allowed to dissolve. With agitation, the required amounts of maltitol powder distributed by Roquette America, Inc., Keokuk, Iowa, maltitol syrup distributed by AlGroup Lonza, Fair Lawn, New Jersey, fructooligosaccharides distributed by Golden Technologies Company, Golden, Colorado, U.S.A. and a second maltodextrin distributed by Matsutani Chemical Industry Co., Hyogo, Japan under the product name Fibersol® 2(E) are added and agitated well until completely dissolved. The required amount of tricalcium phosphate and magnesium phosphate are added to the slurry under agitation. The completed carbohydrate/mineral slurry is held with agitation at a temperature from about 65°C to about 71°C for not longer than twelve hours until it is blended with the other slurries.

A fiber in oil slurry is prepared by combining and heating the required amounts of high oleic safflower oil and canola oil to a temperature from about 40.5°C to about 49°C with agitation. With agitation, the required amounts of lutein esters from Cognis of LaGrange, Illinois is added. Agitate for a minimum of 15 minutes. With agitation, the required amounts of the following ingredients are added to the heated oil: lecithin (distributed by Central Soya Company, Fort Wayne, Indiana), Vitamin D, E, K premix (distributed by Vitamins Inc., Chicago, Illinois), vitamin A, vitamin E and beta-carotene. The required amounts of soy polysaccharide distributed by Protein Technology International, St. Louis, Missouri is slowly dispersed into the heated oil . The completed oil/fiber slurry is held under moderate agitation at a temperature from about 55°C to about 65°C for a period of no longer than twelve hours until it is blended with the other slurries.

A first protein in water slurry is prepared by heating 293 kg of water to 60°C to 65°C. With agitation, the required amount of 20% potassium citrate solution is added and held for one minute. The required amount of acid casein is added under high agitation followed immediately by the required amount of 20% sodium hydroxide. The agitation is maintained at high until the casein is dissolved. The slurry is held from about 60°C to 65°C with moderate agitation.

A second protein in water slurry is prepared by first heating about 77 kg of water to a temperature of about 40°C with agitation. The caseinate is added and the slurry is agitated well until the caseinate is completely dispersed. With continued agitation, the slurry is slowly warmed to 60°C to 65°C. The slurry is held for no longer than twelve hours until it is blended with the other slurries.

The batch is assembled by blending 344 kg of protein slurry one with 84 kg of protein slurry two. With agitation, the 37 kg of the oil/fiber slurry is added. After waiting for at least one minute, 216 kg of the carbohydrate/mineral slurry is added to the blended slurry from the preceding step with agitation and the resultant blended slurry is maintained at a temperature from about 55°C to about 60°C. The pH of the blended batch is adjusted to a pH of 6.45 to 6.75 with 1N potassium hydroxide.

After waiting for a period of not less than one minute nor greater than two hours, the blend slurry is subjected to deaeration, ultra-high-temperature treatment, and homogenization. The blended slurry is heated to a temperature from about 71°C to about 82°C and deaerated under vacuum. The heated slurry is then emulsified through a single stage homogenizer at 900 to 1100 psig. After emulsification , the slurry is heated from about 99°C to about 110°C and then heated to a temperature of about 146°C for about 5 seconds. The slurry is passed through a flash cooler to reduce the temperature to from about 99°C to about 110°C and then through a plate cooler to reduce the temperature to from about 71°C to about 76°C. The slurry is then homogenized at 3900 to 4100/ 400 to 600 psig. The slurry is held at about 74°C to about 80°C for 16 seconds and then cooled to 1°C to about 7°C. At this point, samples are taken for microbiological and analytical testing. The mixture is held under agitation.

A water soluble vitamin (WSV) solution is prepared separately and added to the processed blended slurry.

The vitamin solution is prepared by adding the following ingredients to 9.4 kg of water with agitation: WSV premix (distributed by J.B. Laboratories, Holland, Michigan), vitamin C, choline chloride, L-carnitine, taurine, inositiol, folic acid, pyridoxine hydrochloride and cyanocobalamin. The required amount of 45% potassium hydroxide slurry is added to bring the pH to between 7 and 10.

Based on the analytical results of the quality control tests, an appropriate amount of water is added to the batch with agitation to achieve desired total solids. Additionally, 8.8 kg of vitamin solution is added to the diluted batch under agitation.
The product pH may be adjusted to achieve optimal product stability. The completed product is then placed in suitable containers and subjected to terminal sterilization.

Example VII

Composition of a Pediatric Nutritional Product

Table 3 presents a bill of materials for manufacturing 771 kg of a pediatric enteral nutritional of the instant invention. A detailed description of its manufacture follows.

Table 3: Bill of materials for vanilla pediatric nutritional

Ingredient	Quantity per 771 kg
Stock PIF Slurry	
High oleic safflower oil	40.7 kg
Soy oil	24.4 kg
MCT oil	16.3 kg
Lecithin	840.2 g
Monoglycerides	840.2 g
Carrageenan	508.9 g
Caseinate	32.8 kg
Stock OSV blend	
DEK premix	83.3 g
Vitamin A	7.1 g
Lutein esters (5%)	92 g
Stock PIW slurry	
Water	530 kg
Caseinate	11.3 kg
Whey protein	11.9 kg
Stock MIN slurry	
Water	18 kg
Cellulose gum	1696 g
Calcium HMB monohydrate	4.4kg
Magnesium chloride	2.7 kg
Potassium chloride	1.0 kg
Potassium citrate	2.7 kg
Potassium iodide	0.25g
Dipotassium phosphate	1.45 kg
Final blend	
PIW slurry	251 kg
PIF slurry	53 kg
MIN slurry	12.6 kg
Sodium chloride	127.4 g
Sucrose	77.6 kg

Tricalcium phosphate	2.5 kg
Water	167 kg
Stock WSV solution	
Water	31.7 kg
Potassium citrate	3.74 g
UTM/TM premix	172.2 g
WSV premix	134.1 g
m-inositol	176.7 g
Ttaurine	145.5 g
L-carnitine	34.92 g
Choline chloride	638.7 g
Stock ascorbic acid solution	
Water	18.6 kg
Ascorbic acid	550.0 g
45% KOH	341 g
Stock vanilla solution	
Water	38.5 kg
Vanilla flavor	4.3 kg

DEK premix: (per gm premix) 12,100 IU vitamin D_3, 523 IU vitamin E, 0.962 mg vitamin K_1
UTM/TM premix: (per gm premix) 132 mg zinc, 147 mg iron, 10.8 mg manganese, 12.5 mg copper, 0.328 mg selenium, 0.284 mg molybdenum
WSV premix: (per gm premix) 375 mg niacinamide, 242 mg d-calcium pantothenate, 8.4 mg folic acid, 62 mg thiamine chloride hydrochloride, 48.4 mg riboflavin, 59.6 mg pyridoxine hydrochloride, 165.5 mcg cyanocobalamin, 7305 mcg biotin

The stock oil soluble vitamin blend (OSV blend) is prepared by weighing out the specified amount of DEK premix into a screw cap, light protected container large enough to hold 54 g of oil soluble vitamins. Using a plastic pipette, the required amount of vitamin A is added to the DEK aliquot. The container is flushed with nitrogen prior to applying the lid.

The stock protein in fat slurry (PIF) was prepared by adding the required amounts of high oleic safflower oil, soy oil and MCT oil to the blend tank. The mixture is heated to 40.5°C to 49°C with agitation. With agitation, the required amounts of lutein esters from American River Nutrition of Hadley, Massachusetts is added. Agitate for a minimum of 15 minutes. The emulsifiers, lecithin (distributed by Central Soya of Decatur, Indiana) and monoglycerides (distributed by Quest of Owings Mills, Maryland), are added and mixed well to dissolve. All of the OSV blend is then added. The containers are rinsed out 4 to 5 times with the oil blend to assure complete transfer of the vitamins. The carrageenan (distributed by FMC of Rockland, Maine) and the caseinate are added. The slurry is

mixed well to disperse the protein. The PIF slurry is held up to six hours at 60 - 65°C under moderate agitation until used.

The stock protein in water slurry (PIW) is prepared by adding the required amount of water to a blend tank. The water is held under moderate agitation and brought up to 76 - 82°C. The required amount of caseinate is added to the water under high agitation and mixed on high until the protein is fully dispersed. The protein slurry is allowed to cool to 54 - 60°C before proceeding. Once cooled the required amount of whey protein is added and mixed well until fully dispersed/dissolved. The PIW slurry is held up to two hours at 54 - 60°C until used.

The stock mineral solution (MIN) is prepared by adding the required amount of water to a blend tank and heated to 60 - 68°C. The cellulose gum blend (distributed by FMC of Newark, Delaware) is added to the water and held under moderate agitation for a minimum of five minutes before proceeding. The calcium HMB is added and agitated for a minimum of five minutes before proceeding. The mineral salts magnesium chloride, potassium chloride, potassium citrate, potassium iodide and dipotassium phosphate are added one at a time with mixing between each addition to ensure the minerals dissolved. The completed MIN solution is held at 54 - 65°C under low to moderate agitation until used.

The final blend is prepared by adding the specified amount of PIW slurry to a blend tank and heated under agitation to 54 -60°C. The specified amount of PIF slurry is added to the tank and mixed well. The specified amount of MIN solution is added to the blend and mixed well. The specified amount of sodium chloride is added to the blend and mixed well. The specified amount of sucrose is added to the blend and mixed well to dissolve. The tricalcium phosphate is added to the blend and mixed well to disperse. The specified amount of additional water is added to the blend and mixed well. The completed final blend is held under continuous agitation at 54 - 60°C. If necessary, the pH is adjusted to 6.45 - 6.8 with 1N KOH.

After waiting for a period of not less than one minute nor greater than two hours, the blend slurry is subjected to deaeration, ultra-high-temperature treatment, and homogenization. The blended slurry is heated to a temperature from about 68°C to about 74°C and deaerated under vacuum. The heated slurry is then emulsified at 900 to 1100 psig. After emulsification , the slurry is heated from about 120°C to about 122°C and then heated to a temperature of about 149°C to about 150°C. The slurry is passed through a flash cooler to reduce the temperature to from about 120°C to about 122°C and then through a plate cooler to reduce the temperature to from about 74°C to about 79°C. The slurry is then homogenized at 3900 to 4100/ 400 to 600 psig. The slurry is held at about 74°C to about 85°C for 16 seconds and then cooled to 1°C to about 6°C. At this point,

samples are taken for microbiological and analytical testing. The mixture is held under agitation.

Standardization proceeds as follows. The stock vitamin solution (WSV) is prepared by heating the specified amount of water to 48 - 60°C in a blend tank. Potassium citrate, UTM/TM premix (distributed by Fortitech of Schenectady, New York), WSV premix, m-inositol, taurine, L-carnitine and choline chloride are each added to the solution in the order listed and allowed to mix well to dissolve or disperse each ingredient. 14.2 kg of the vitamin solution is added to the processed mix tank.

The stock vanilla solution is prepared by adding the specified amount of water to a blend tank. The specified amount of vanilla (distributed by Givaudan Roure of Cincinnati, Ohio) is added to the water and mixed well. 18.5 kg of vanilla solution is added to the processed mix tank and mixed well.

The stock ascorbic acid solution is prepared by adding the required amount of water to a blend tank. The specified amount of ascorbic acid is added and mixed well to dissolve. The specified amount of 45% KOH is added and mixed well. 8.4 kg of ascorbic acid solution is added to the mix tank and mixed well.

The final mix is diluted to the final total solids by adding 92.5 kg of water and mixed well. Product is filed into suitable containers prior to terminal (retort) sterilization.

Example VIII

Composition of a Complete Nutritional Supplement

Table 4 presents a bill of materials for manufacturing 1,000 kg of a typical vanilla flavored meal replacement liquid. A detailed description of its manufacture follows.

Table 4: Bill of Materials for Vanilla Liquid Nutritional

Ingredient	Quantity per 1,000kg
Water	QS
Corn Syrup	33 kg
Maltodextrin	28 kg
Sucrose	19.4 kg
Caseinate	8.7 kg
Calcium HMB monohydrate	5.7 kg
High Oleic Safflower Oil	4.1 kg
Canola Oil	4.1 kg
Soy Protein	3.7 kg
Whey Protein	3.2 kg
Caseinate	2.9 kg
Corn Oil	2.0 kg
Tricalcium Phosphate	1.4 kg
Potassium Citrate	1.3 kg
Magnesium Phosphate	952 gm

Lecithin	658 gm
Magnesium chloride	558 gm
Vanilla Flavor	544 gm
Sodium Chloride	272 gm
Carrageenan	227 gm
Choline chloride	218 gm
UTM/TM Premix	165 gm
Potassium Chloride	146 gm
Ascorbic Acid	145 gm
Sodium Citrate	119 gm
Potassium Hydroxide	104 gm
Lutein (5%)	46 gm
WSV Premix	33 gm
Vit DEK Premix	29 gm
Vitamin A	3.7 gm
Potassium Iodide	86 mcg

WSV premix(per g premix): 375 mg/g niacinamide, 242 mg/g calcium pantothenate, 8.4 gm/g folic acid, 62 mg/g thiamine chloride hydrochloride, 48.4 gm/g riboflavin, 59.6 mg/g pyridoxine hydrochloride, 165 mcg/g cyanocobalamin and 7305 mcg/g biotin
Vitamin DEK premix(per g premix): 8130 IU/g vitamin D_3, 838 IU/g vitamin E, 1.42 mg/g vitamin K_1
UTM/TM premix(per g premix): 45.6 mg/g zinc, 54 mg/g iron, 15.7 manganese, 6.39 mg/g copper, 222 mcg/g selenium, 301 mcg/g chromium and 480 mcg/g molybdenium

The liquid meal replacement products of the present invention are manufactured by preparing three slurries that are blended together, heat treated, standardized, packaged and sterilized.

A carbohydrate/mineral slurry is prepared by first heating the required amount of water to a temperature of from about 65°C to about 71°C with agitation. The required amount of calcium HMB is added and agitated for a minimum of 5 minutes. With agitation, the required amount of potassium citrate and ultra trace mineral/trace mineral (UTM/TM) premix (distributed by Fortitech, Schnectady, New York) is added. The slurry is greenish yellow in color. Agitation is maintained until the minerals are completely dispersed. With agitation, the required amounts of the following minerals are then added: magnesium chloride, potassium chloride, sodium chloride, sodium citrate, potassium iodide, magnesium phosphate and tricalcium phosphate. Next, the maltodextrin distributed by Grain Processing Corporation, Muscataine, Iowa, U.S.A., sucrose and corn syrup are added to slurry under high agitation, and are allowed to dissolve. The completed carbohydrate/mineral slurry is held with agitation at a temperature from about 65°C to about 71°C for not longer than eight hours until it is blended with the other slurries.

A protein in fat slurry (PIF) is prepared by combining and heating the required amounts of high oleic safflower oil and canola oil to a temperature from about 40.5°C to about 49°C with agitation. With agitation, the required amounts of free lutein from Kemin

Foods of Des Moines, Iowa is added. Agitate for a minimum of 15 minutes. Add the following ingredients are added to the heated oil: lecithin (distributed by Central Soya Company, Fort Wayne, Indiana), vitamin A, and Vitamin D, E, K premix (distributed by Vitamins Inc., Chicago, Illinois). The required amount of carrageenan is dry blended with the required amount of whey protein and add to the agitating lipid mixture and allowed to agitate for a minimum of 10 minutes. The required amount of soy protein is added to the blend slowly to assure proper mixing. The completed oil/protein slurry is held under moderate agitation at a temperature from about 40°C to about 43°C for a period of no longer than two hours until it is blended with the other slurries.

A protein in water slurry is prepared by first heating about required amount of water to a temperature of about 40°C with agitation. The caseinate is added and the slurry is agitated well until the caseinate is completely dispersed. With continued agitation, the slurry is slowly warmed to 60°C to 65°C. The slurry is held for no longer than twelve hours until it is blended with the other slurries.

The batch is assembled by blending required amount of protein slurry with required amount of the carbohydrate/mineral slurry and allowed to agitate for 10 minutes. With agitation, the required amount of the oil/protein slurry is added and agitate for at least 10 minutes. The pH of the blended batch is adjusted to a pH of 6.66 to 6.75 with 1N potassium hydroxide.

After waiting for a period of not less than one minute nor greater than two hours, the blend slurry is subjected to deaeration, ultra-high-temperature treatment, and homogenization. The blended slurry is heated to a temperature from about 71°C to about 82°C and deareated under vacuum. The heated slurry is then emulsified through a single stage homogenizer at 900 to 1100 psig. After emulsification, the slurry is heated from about 99°C to about 110°C and then heated to a temperature of about 146°C for about 5 seconds. The slurry is passed through a flash cooler to reduce the temperature to from about 99°C to about 110°C and then through a plate cooler to reduce the temperature to from about 71°C to about 76°C. The slurry is then homogenized at 3900 to 4100/ 400 to 600 psig. The slurry is held at about 74°C to about 80°C for 16 seconds and then cooled to 1°C to about 7°C. At this point, samples are taken for microbiological and analytical testing. The mixture is held under agitation.

A water soluble vitamin (WSV) solution is prepared separately and added to the processed blended slurry.

The vitamin solution is prepared by adding the following ingredients to 9.4 kg of water with agitation: WSV premix (distributed by J.B. Laboratories, Holland, Michigan), vitamin C, choline chloride, L-carnitine, taurine, inositiol, folic acid, pyridoxine

hydrochloride and cyanocobalamin. The required amount of 45% potassium hydroxide slurry is added to bring the pH to between 7 and 10.

Based on the analytical results of the quality control tests, an appropriate amount of water is added to the batch with agitation to achieve the desired total solids. Additionally, 8.8 kg of vitamin solution is added to the diluted batch under agitation.

The product pH may be adjusted to achieve optimal product stability. The completed product is then placed in suitable containers and subjected to terminal sterilization.

Example IX

Composition of a Beverage

To produce a 1000 kg batch of ready-to-drink beverage, 987.31 kg of water is placed in a vessel fitted with an agitator. At ambient temperature, the requried amount of potassium benzoate is added and allowed to completely dissolve. The reuqired amount of calcium HMB is added and allowed to completely dissolve. The following ingredients are then added in the order listed. Each ingredient is completely dissolved before the next ingredient is added.

Table 5: Ready-to-drink beverage

Potassium benzoate	0.30	kg
Calcium HMB monohydrate	5.7	kg
Potassium Citrate	0.15	kg
Citric Acid	2.89	kg
Lactic Acid	1.41	kg
Aspartame	0.55	kg
Calcium Glycerophosphate	6.06	kg
Coloring Agents	0.0019	kg
Natural and artificial flavors	1.00	kg
Ascorbic acid	0.33	kg

The ascorbic acid was added just before filling into 12-oz. aluminum cans.

The beverages may be carbonated prior to filling into aluminum cans. The solution is de-aerated and then transferred to a "carbo-cooler" where it is cooled and carbonated to approximately 2.5 volumes of carbon dioxide.

EXAMPLE X

Composition of an Electrolyte Replacement Product

The following example explains how to manufacture a ready-to-drink rehydration solution. The ORS had the composition outlined in Table 6.

TABLE 6: Ready-to-drink Rehydration Solution

Ingredient	Quantity per 454 kg
Water	437 kg
Dextrose, Monohydrate	10 kg
Fructose	2.4 kg
Citric Acid	1.2 kg
Sodium Chloride	0.937 kg
Potassium Citrate	1 kg
Sodium Citrate	492.0 g
Calcium HMB monohydrate	5.7 kg
Fruit Flavor	226.8 g
Zinc Gluconate	80.62 g
Sucralose	179.2 g
Acesulfame Potassium	38.1 g
Yellow #6	7.2 g

Weigh out the required amount of filtered water and add to blend tank. Heat the water to 43-54°C, with moderate agitation. While maintaining moderate agitation, the calcium HMB is added and allowed to mix for a minimum of 5 minutes. With continued moderate agitation add the required amount of dextrose. Agitate until dissolved. Add the required amount of fructose. Agitate until dissolved. Add the required amount of the following ingredients, in the order listed, to the dextrose/fructose blend and agitate until dissolved: zinc gluconate, sodium citrate, sodium chloride, potassium citrate, and citric acid. Add the required amount of sucralose (distributred by McNeil Speciality Products Company of New Brunswick, New Jersey) and acesulfame potassium (distributed as Sunsett® by Hoechst Food Ingredients of Somerset, New Jersey) and agitate until dissolved. Add the yellow #6 and the fruit punch flavor to the batch until dissolved. Cool the blend to 1.1-7.2°C and hold with low agitation. Fill the required number of one liter plastic bottles, apply the foil heat seal to the bottle opening, and retort to food grade sterility standards.

Alternatively, the cooled blend is encapsulated within a sealable freezable packaging material and sealed such as by heat sealing. A single dose of rehydration solution is packaged in a hermetically sealed freezable pouch. Various types of packaging materials which can be used to practice the invention, such as that used in traditional freezer pops, would be readily apparent to the skilled artisan. The wrapping material is preferably a type which will allow markings, such as product identification, ingredients, etc., to be placed on the exterior surface thereof. The rehydration formulation is shipped and stored, preferably in multiple units thereof, in this condition. It is contemplated that multiple units or freezer pops will be packaged together for purposes of commercialization.

Prior to administration, a package of liquid rehydration solution is frozen. Following freezing, the package is opened and the contents thereof eaten. Since the frozen rehydration formulation will normally be administered at ambient temperatures, the amount of rehydration liquid contained in each package is preferably an amount which can be consumed in its entirely while still in the frozen state. Preferably 20-35 ounces, more preferably 2.0 to 2.5 ounces per package. In a particularly preferred embodiment, 2.1 ounces of sterile rehydration solution is encapsulated within an rectangular, e.g., 1".times.8," freezable wrapper material. Clear plastic wrapper material is preferred.

THE EMBODIMENTS OF THE INVENTION FOR WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. Use of beta-hydroxy-beta-methylbutyric acid (HMB), its salts, metabolites or derivatives thereof for the manufacture of a product that is substantially lacking in free amino acids, for prevention or treatment a disease-associated wasting condition, wherein the disease-associated wasting condition is selected from the group consisting of cachexia, age-associated wasting, and wasting associated with long-term hospital stay.

2. The use according to claim 1 wherein the product comprising HMB, its salts, metabolites or derivatives thereof downregulates the expression and/or activity of protein kinase C.

3. The use according to claim 1 wherein the product comprising HMB, its salts, metabolites or derivatives thereof downregulates the expression and/or activity of nuclear factor kappa-B.

4. The use according to claim 1 wherein the product comprising HMB, its salts, metabolites or derivatives thereof downregulates the expression and/or activity of ubiquitin-conjugating enzymes.

5. The use according to claim 1 wherein the product comprising HMB, its salts, metabolites or derivatives thereof downregulates the expression and/or activity of components of 26S proteasome.

6. The use according to claim 1 wherein the product further comprises at least one of the components selected from the group consisting of L-carnitine, amino nitrogen source enriched with large neutral amino acids substantially lacking free amino acids, omega-3 fatty acids and indigestible oligossacharide.

7. The use according to claim 1 wherein the disease is cachexia.

8. The use according to claim 1 wherein said product comprises:

   a. HMB, its salts, metabolites or derivatives thereof;

   b. carnitine; and

c. amino nitrogen source enriched with large neutral amino acids.

9. The use according to claim 8 wherein said HMB is selected from the group consisting of sodium HMB, potassium HMB, magnesium HMB, chromium HMB, calcium HMB, alkali metal HMB, alkaline earth metal HMB and HMB lactone.

10. The use according to claim 8 wherein said product further comprises ω-3 fatty acids.

11. The use according to claim 10 wherein said ω-3 fatty acids are selected from the group consisting of eicosapentaenoic acid and docosahexaenoic acid.

12. The use according to claim 8 wherein said large neutral amino acids comprise at least 10% of the amino nitrogen source.

13. The use according to claim 8 wherein said free amino acids comprise less than 0.4 gm/serving of the product.

14. The use according to claim 8 wherein said product comprises less than 2 grams per serving of carnitine.

15. The use according to claim 8 wherein said product further comprises at least 1 gram per serving of FOS.

16. The use according to claim 8 wherein said product further comprises a nutrient selected from the group consisting of vitamins, minerals, and trace minerals.

17. The use according to claim 1 wherein said product comprises:

a. from about 2 to 10 gm/liter calcium HMB;

b. at least 1 gram per liter of ω-3 fatty acids;

c. from about 1 to about 8 gm/liter carnitine;

d. from about 1 to about 25 gm/liter FOS; and

amino nitrogen source enriched with large neutral amino acids, wherein said amino nitrogen source comprises from about 10 to 60 wt/wt % large neutral amino acids.

18. The use according to claim 8 wherein said product is for administration to a human or an animal.

19. The use according to claim 8 wherein said product is selected from the group consisting of dietary supplement, meal replacement, nutritional bars, chews or bites and beverage.

Potential intracellular events in skeletal muscle involved in PIF induced proteasome activation

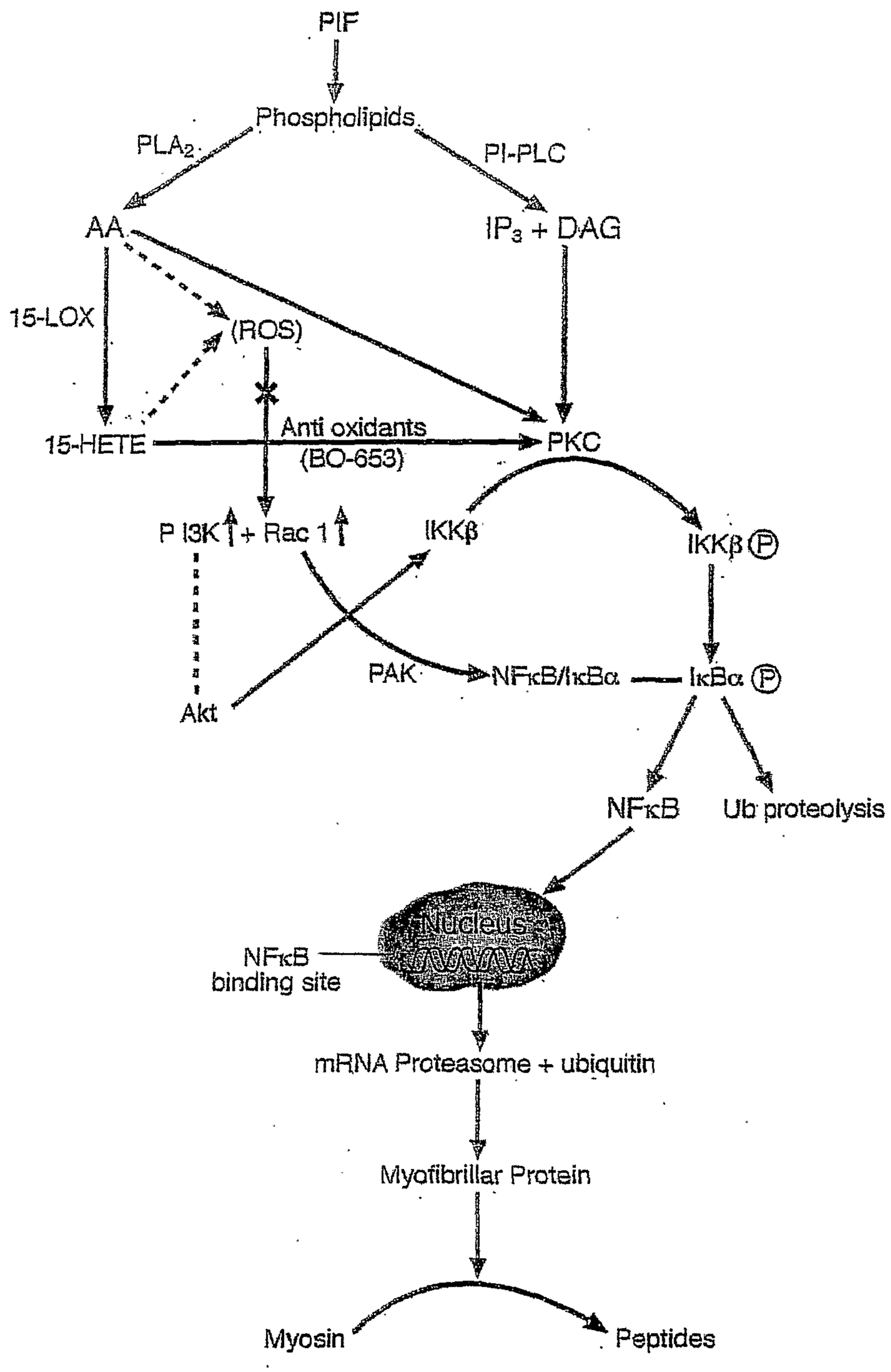
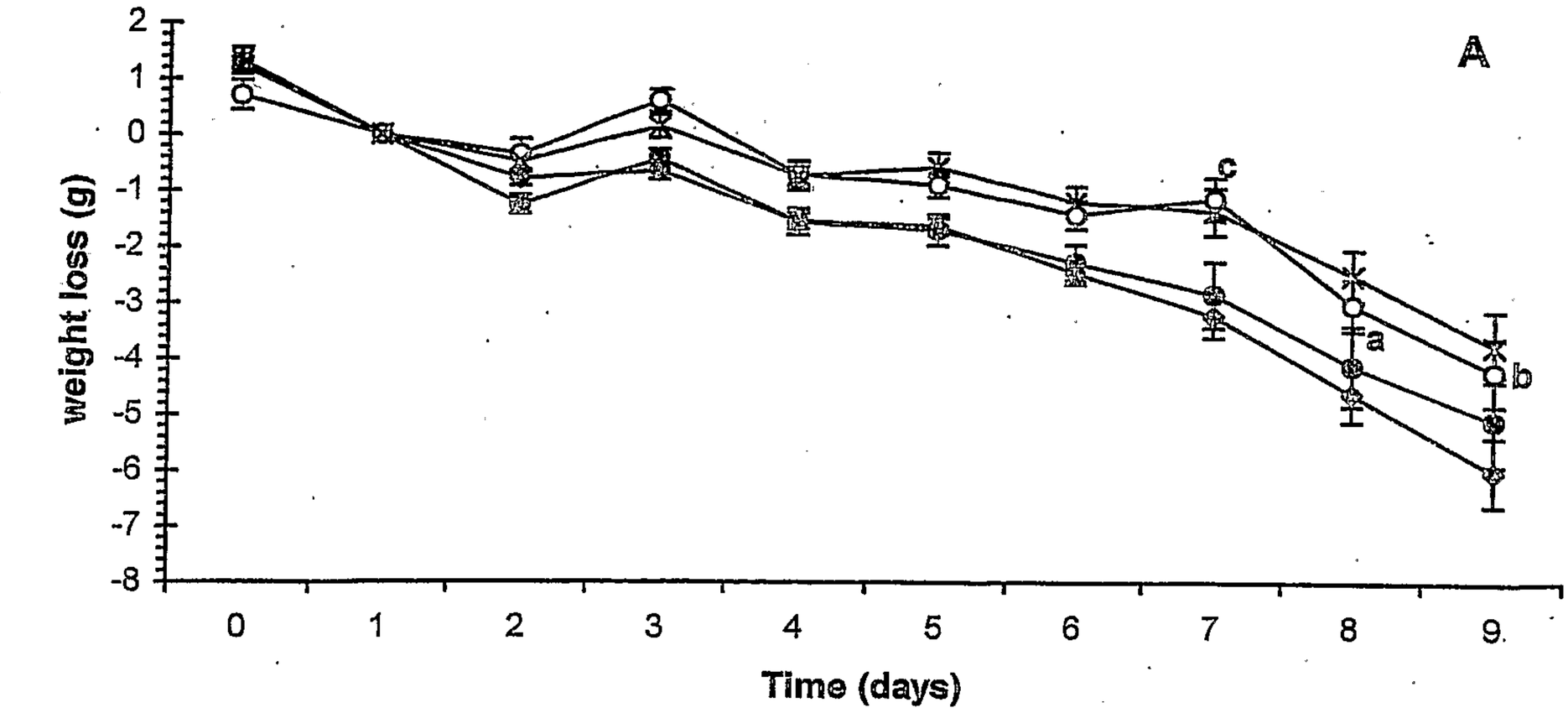


Figure 1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-2
-3
-4
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-6
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1
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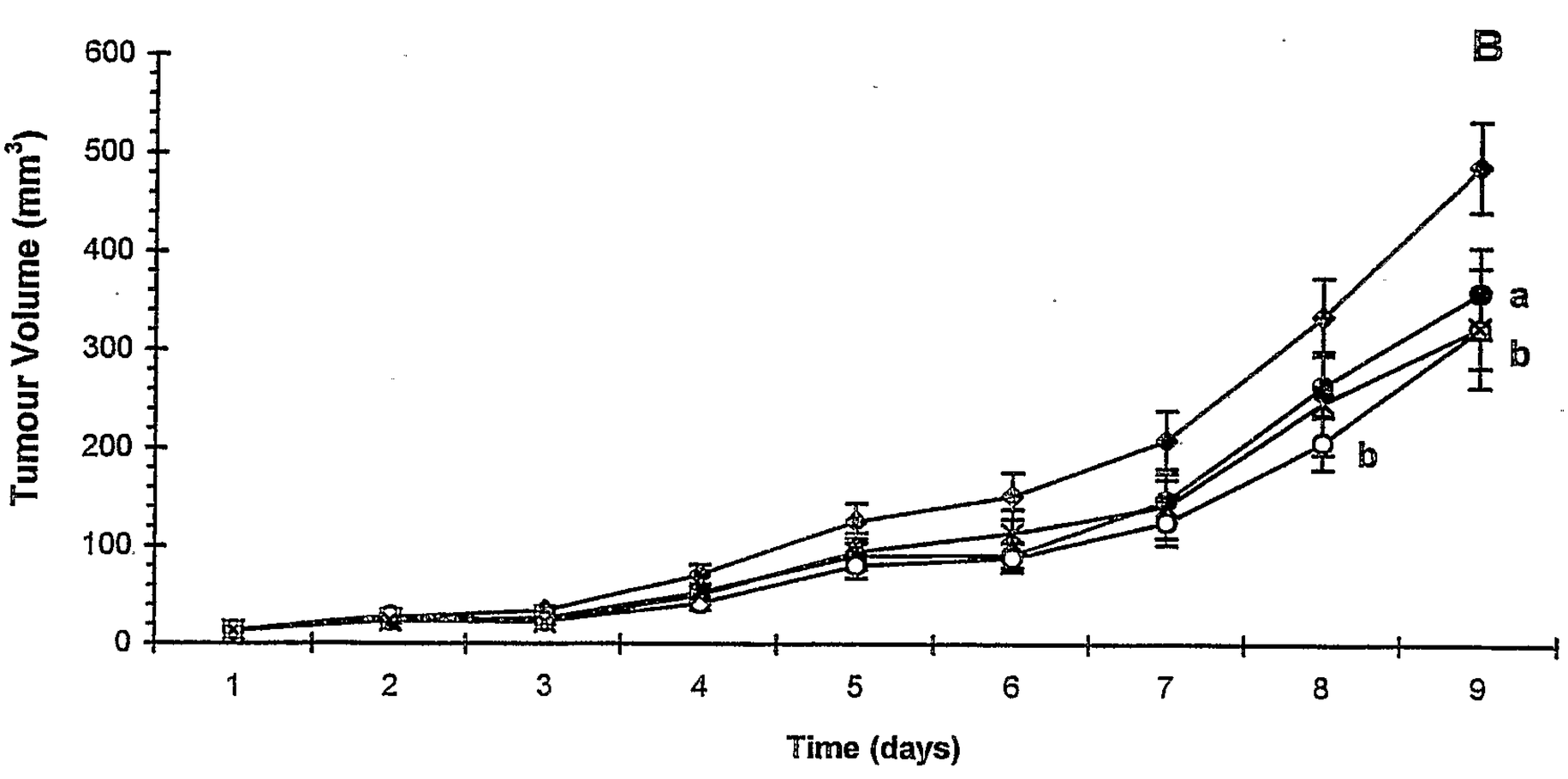
3
4
5
6
7
8
9
Time (days)
a
b
c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500
400
300
200
100
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2
3
4
5
6
7
8
9
Time (days)
a
b
b

Figure 2

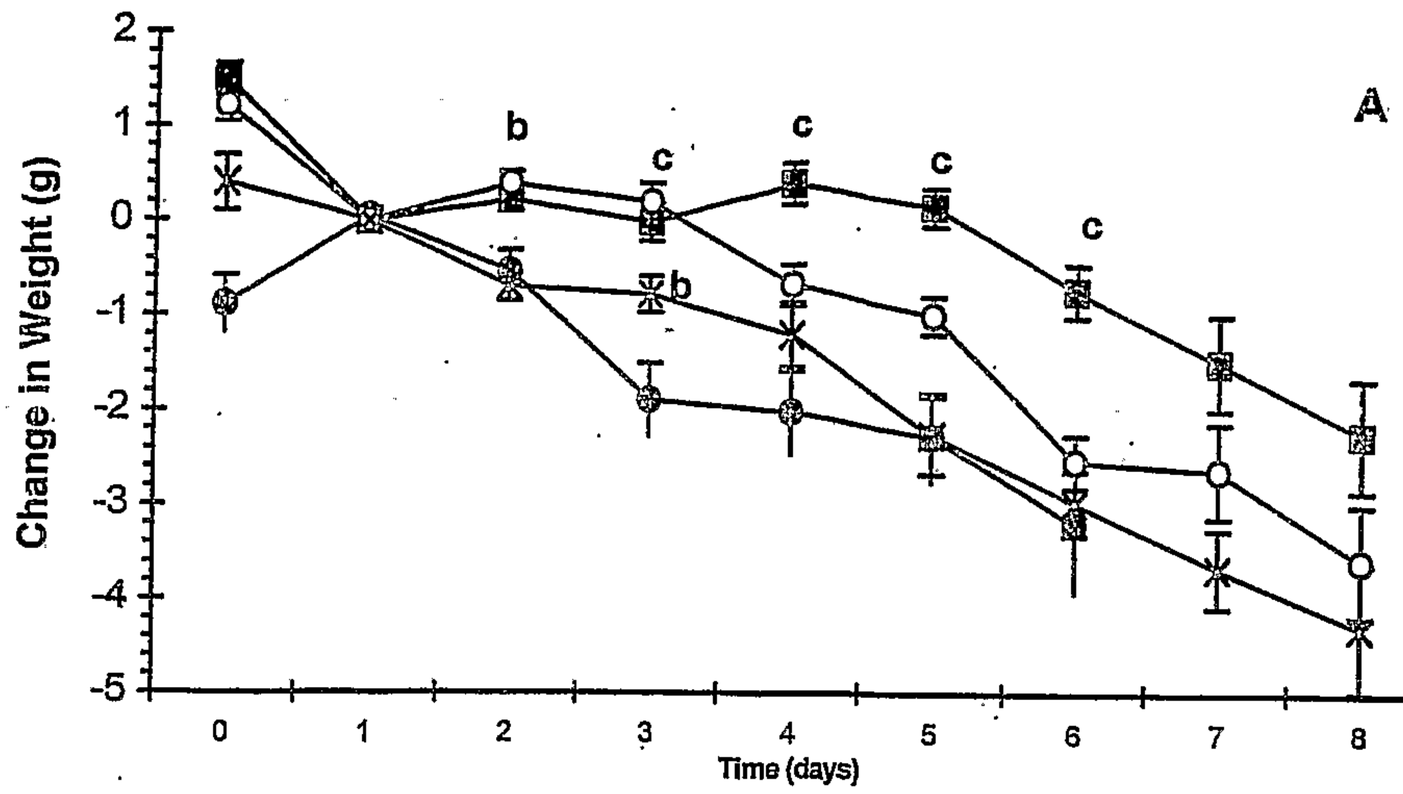
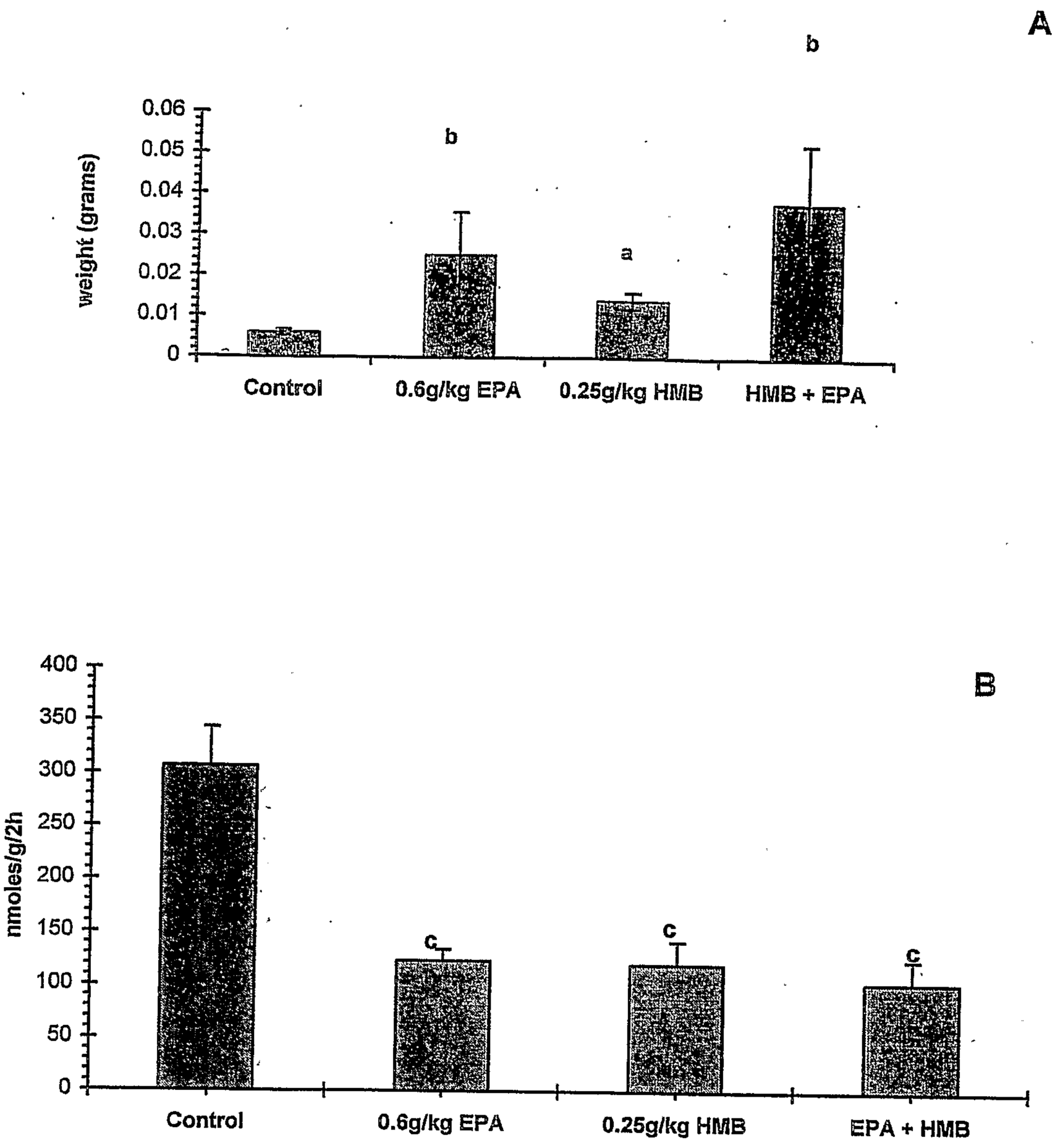


Figure 3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0.04
0.03
0.02
0.01
0
b
a
b
Control
0.6g/kg EPA
0.25g/kg HMB
HMB + EPA
B
nmoles/g/2h
400
350
300
250
200
150
100
50
0
c
c
c
Control
0.6g/kg EPA
0.25g/kg HMB
EPA + HMB

Figure 4

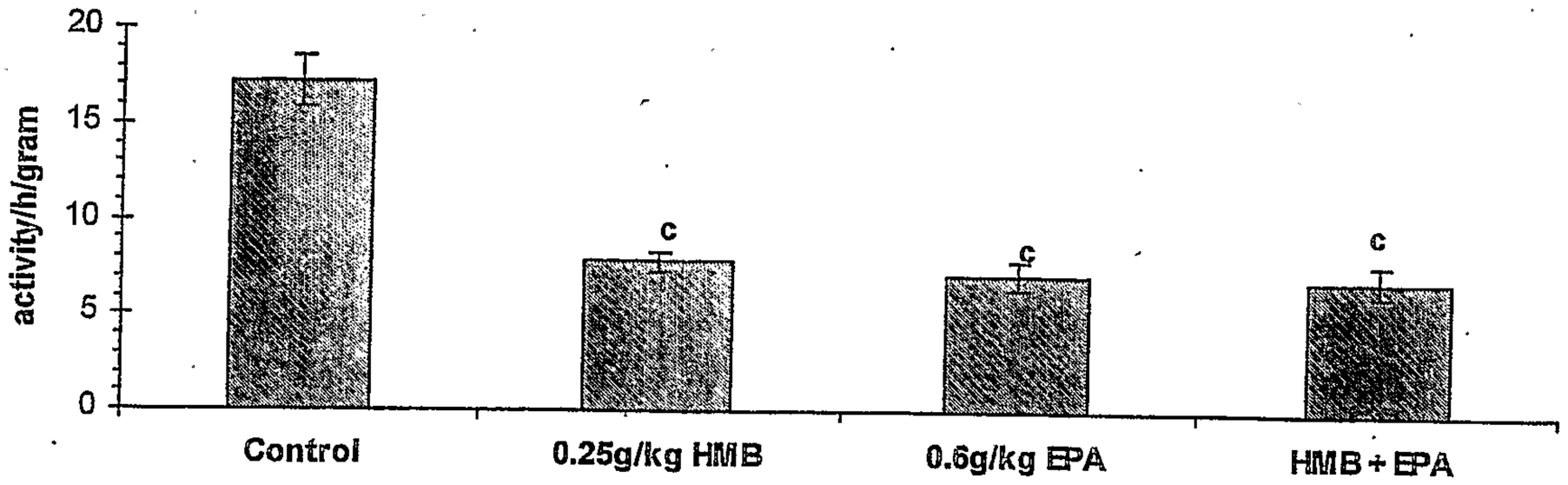
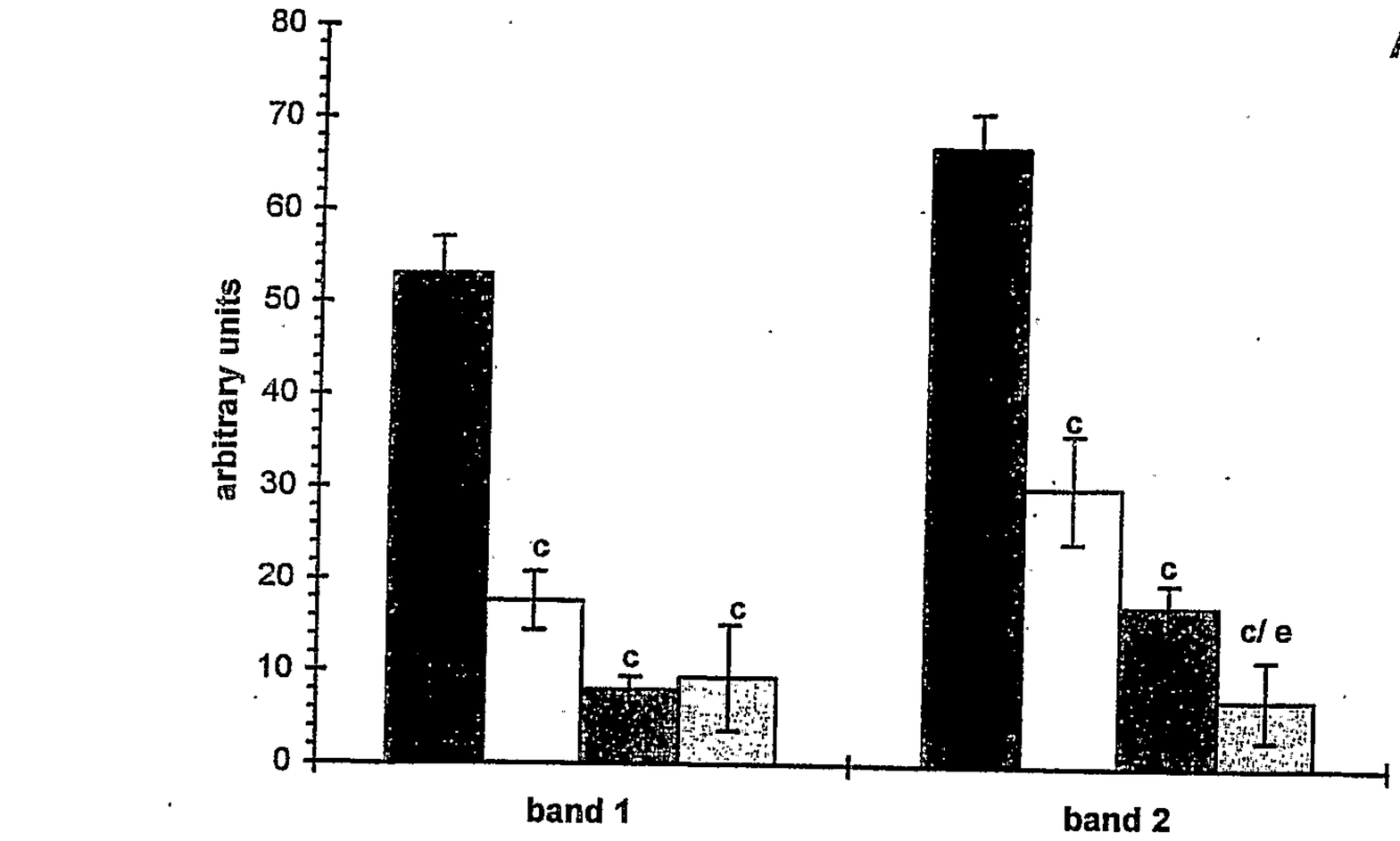

20
15
10
5
0
activity/h/gram
c
c
c
Control
0.25g/kg HMB
0.6g/kg EPA
HMB + EPA

Figure 5


A
arbitrary units
80
70
60
50
40
30
20
10
0
c
c
c
c
c/ e
band 1
band 2

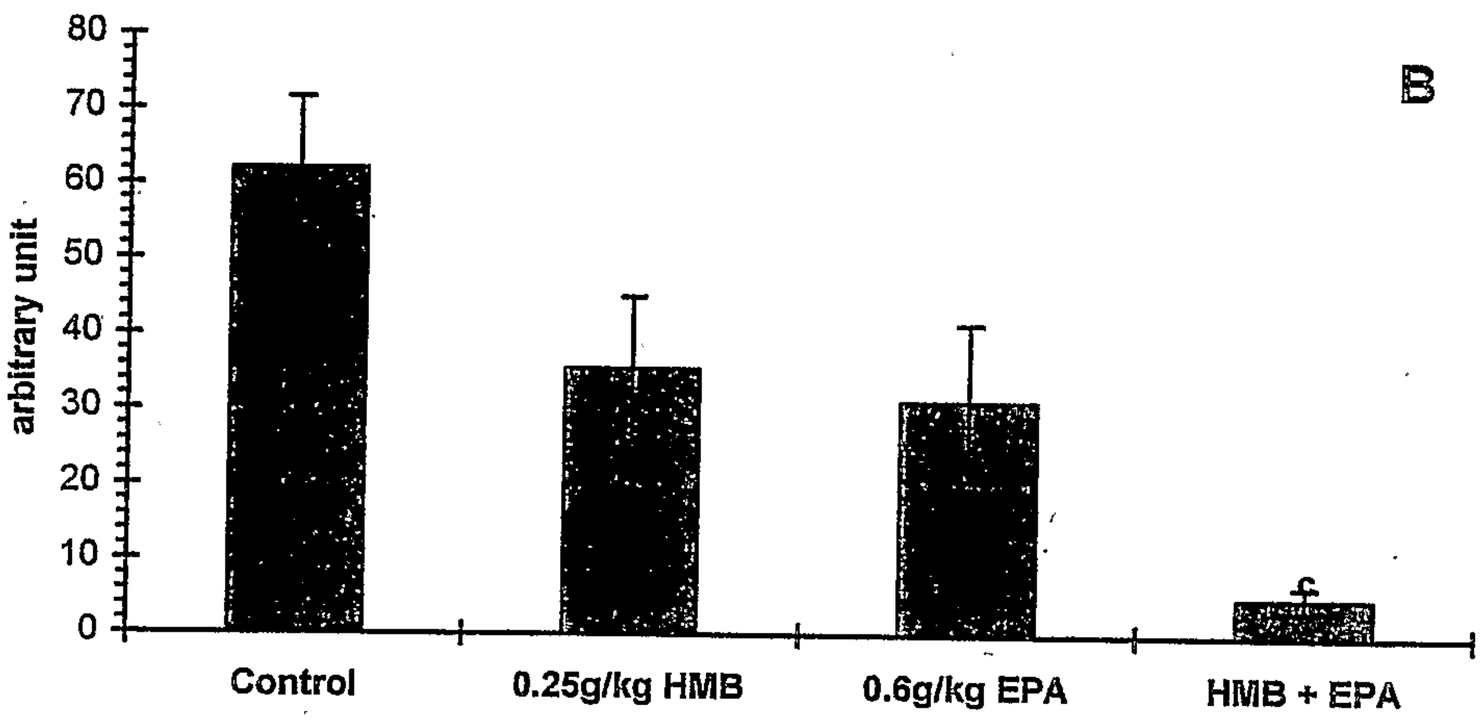

B
arbitrary unit
80
70
60
50
40
30
20
10
0
f
Control
0.25g/kg HMB
0.6g/kg EPA
HMB + EPA

Figure 6

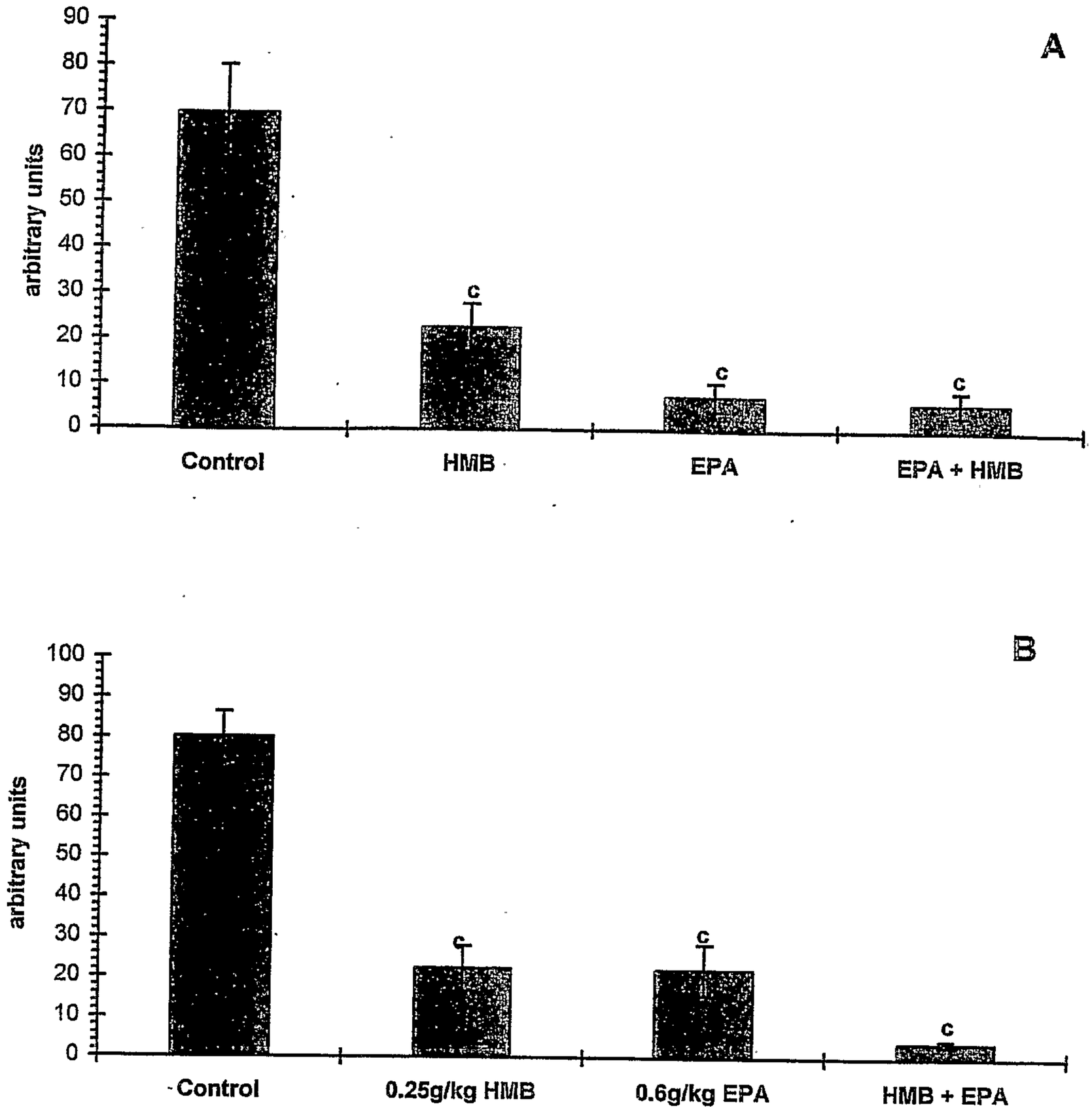

A
arbitrary units
90
80
70
60
50
40
30
20
10
0
c
c
c
Control
HMB
EPA
EPA + HMB
B
arbitrary units
100
90
80
70
60
50
40
30
20
10
0
c
c
c
Control
0.25g/kg HMB
0.6g/kg EPA
HMB + EPA

Figure 7

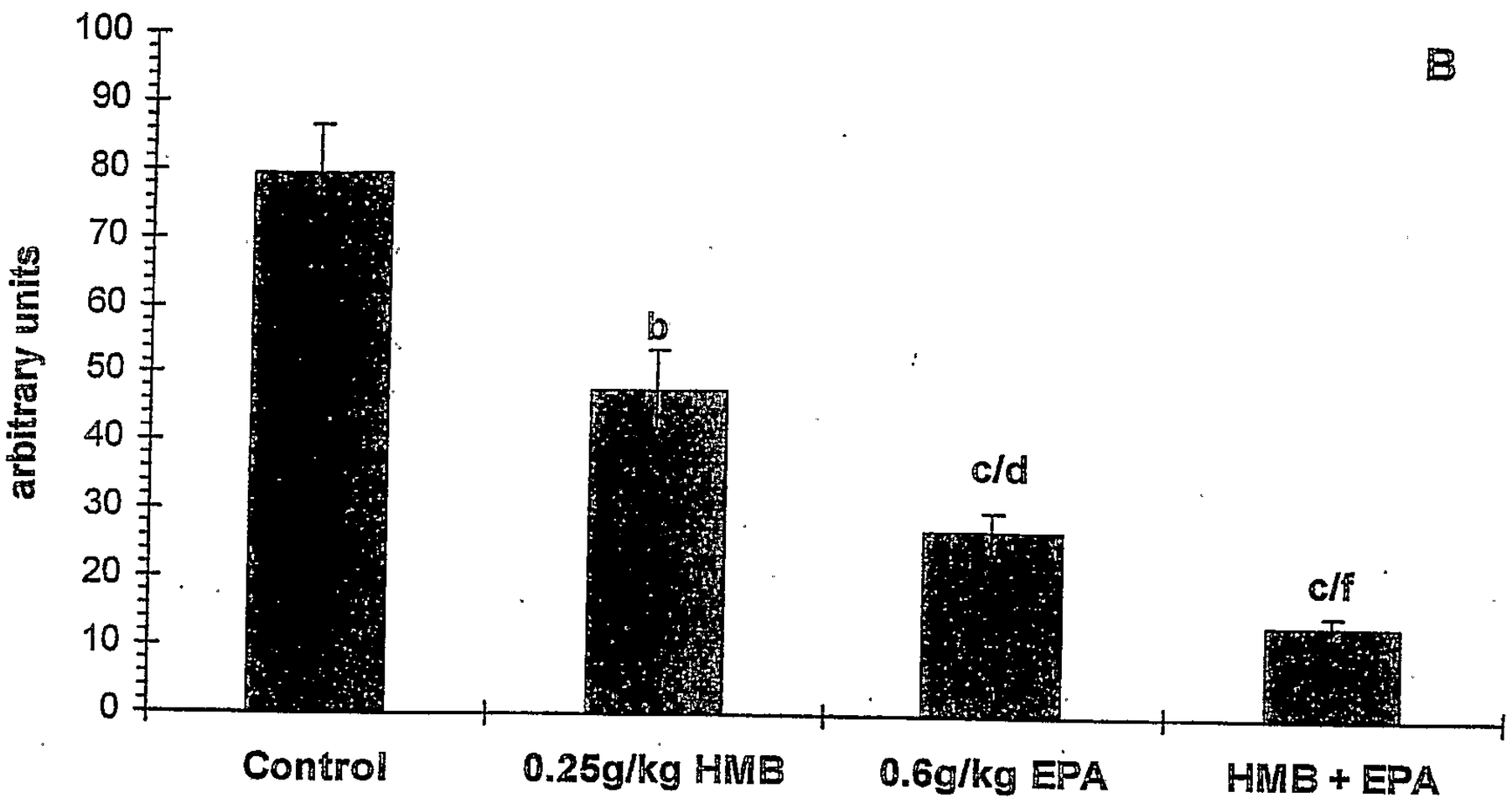
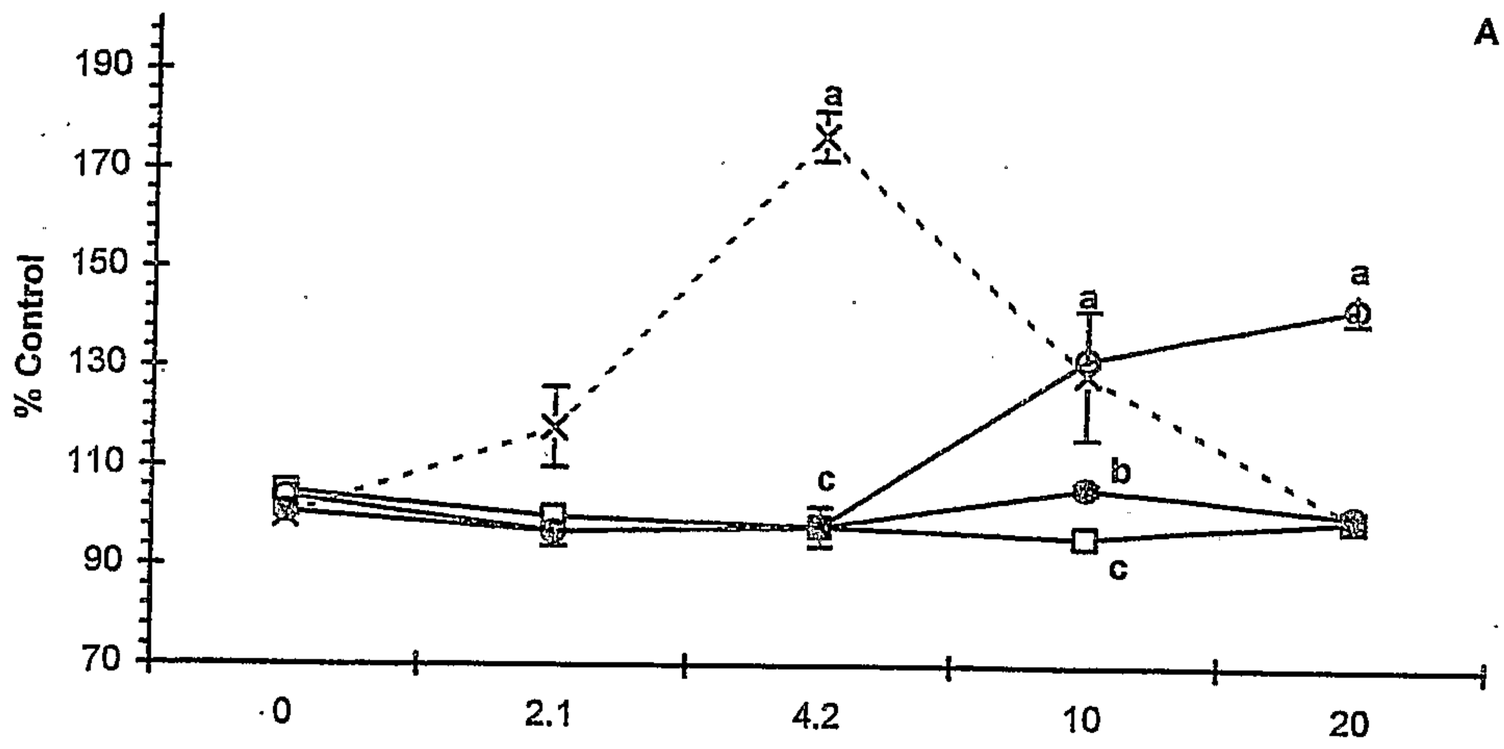

B
arbitrary units
100
90
80
70
60
50
40
30
20
10
0
b
c/d
c/f
Control
0.25g/kg HMB
0.6g/kg EPA
HMB + EPA

Figure 8


A
% Control
190
170
150
130
110
90
70
0
2.1
4.2
10
20
PIF nM
a
b
c

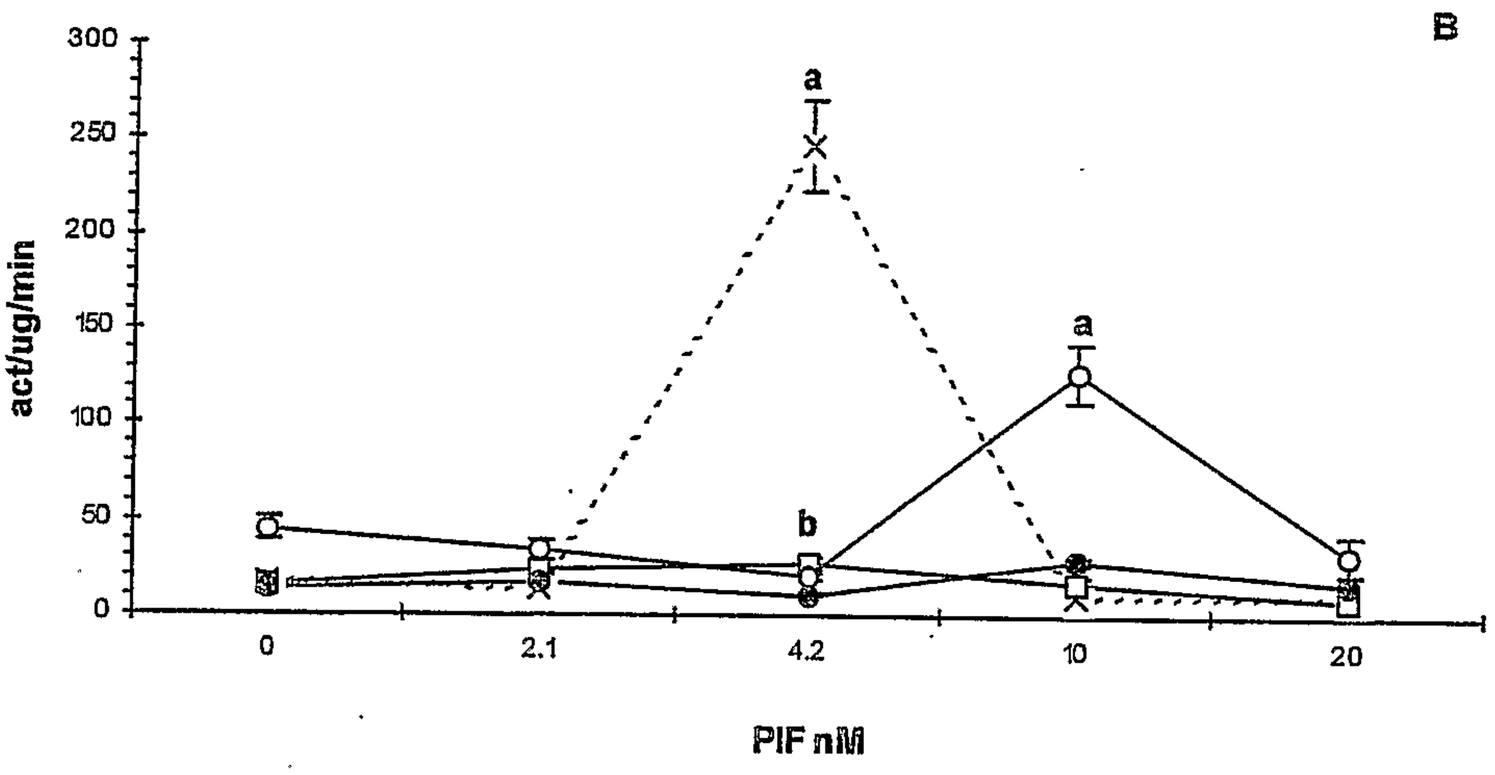

B
act/ug/min
300
250
200
150
100
50
0
0
2.1
4.2
10
20
PIF nM
a
b

Figure 9.

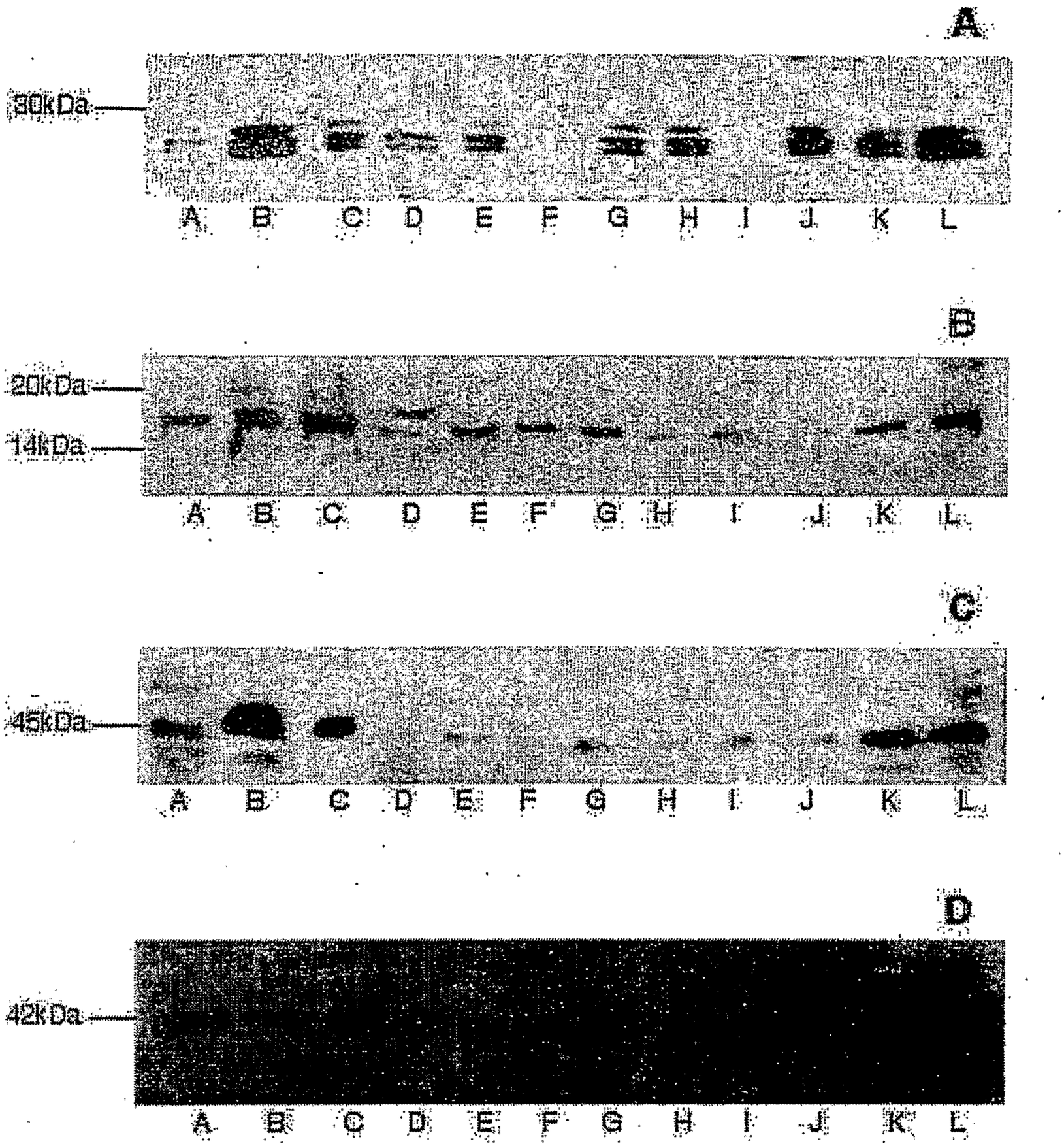

A
30kDa
A B C D E F G H I J K L
B
20kDa
14kDa
A B C D E F G H I J K L
C
45kDa
A B C D E F G H I J K L
D
42kDa
A B C D E F G H I J K L

Figure 10

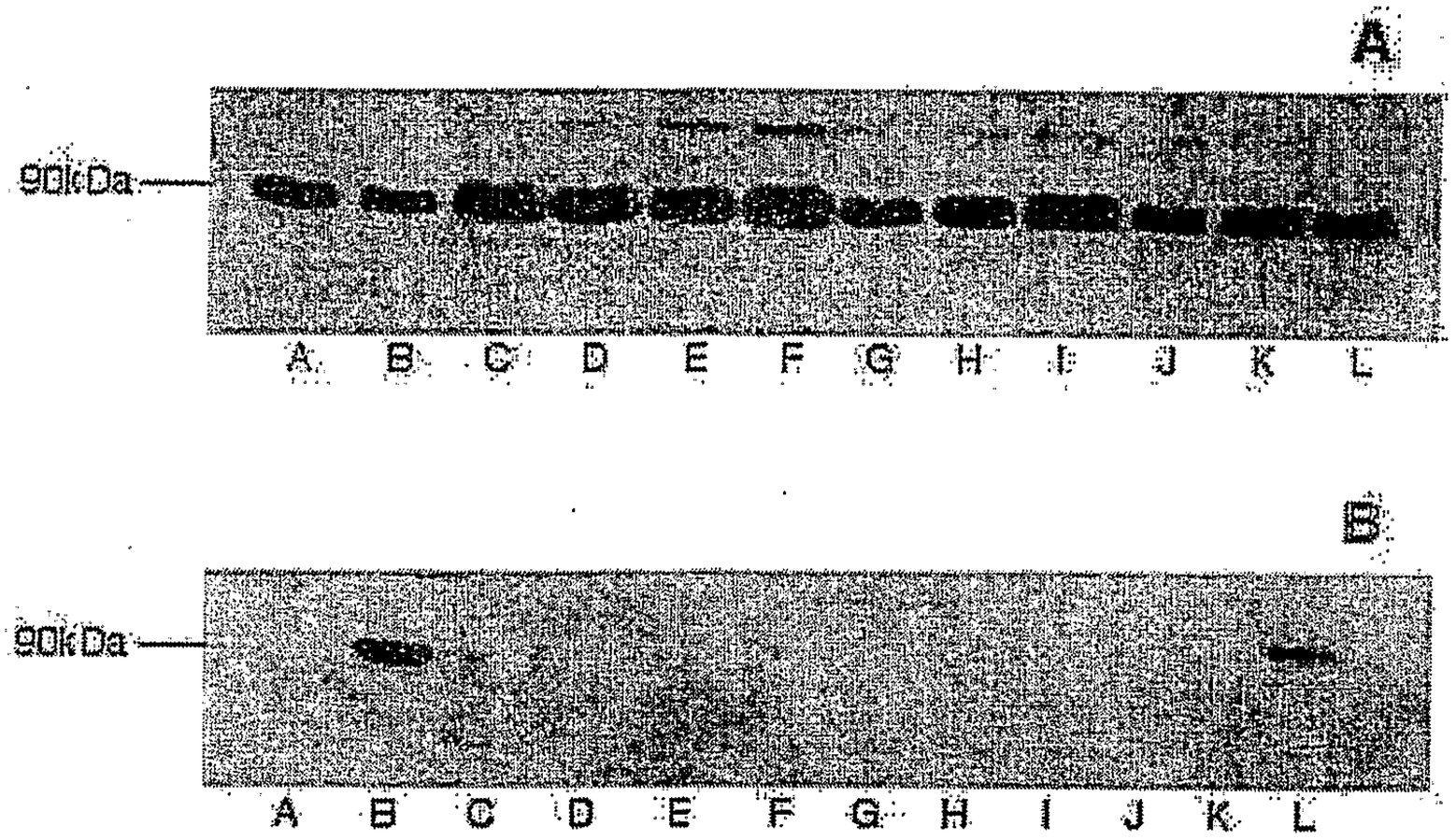
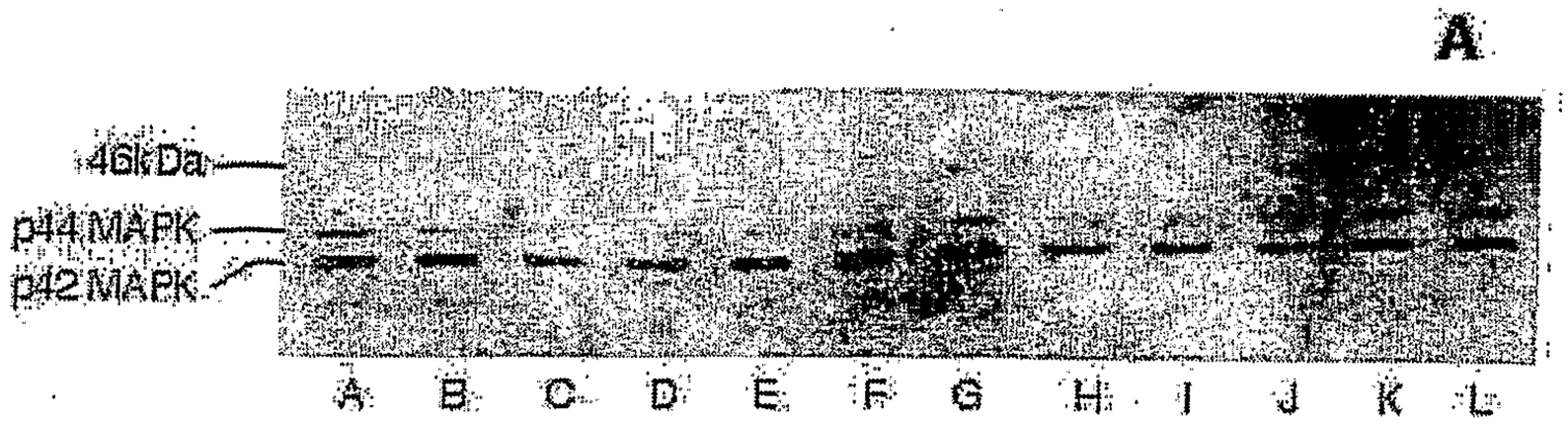


Figure 11


A
46kDa
p44MAPK
p42MAPK
A B C D E F G H I J K L

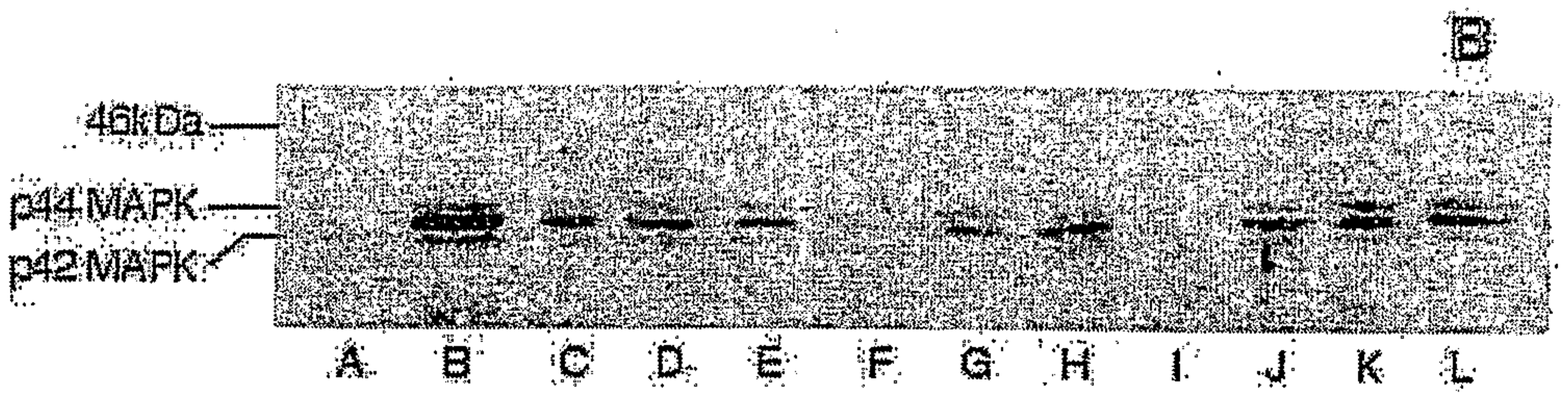
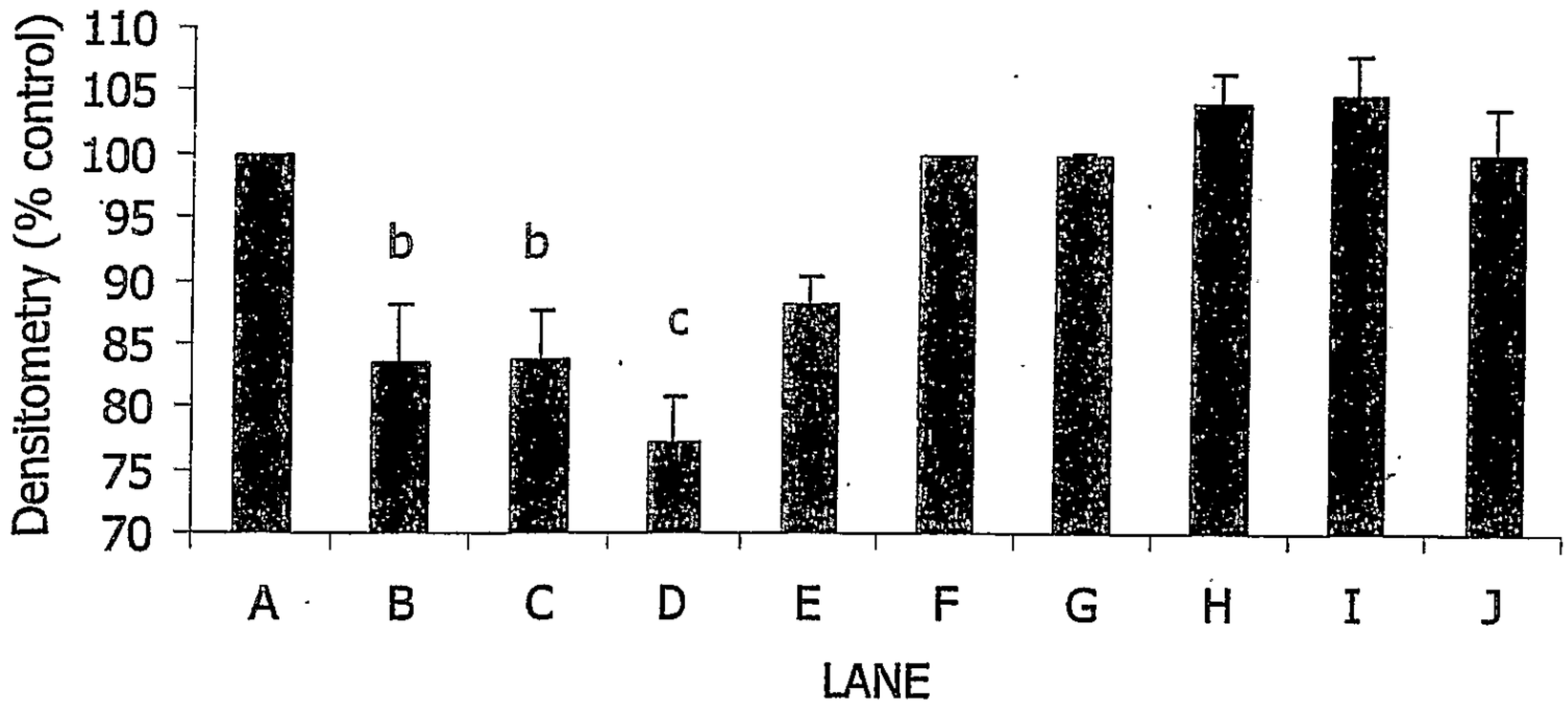

B
46kDa
p44MAPK
p42MAPK
A B C D E F G H I J K L

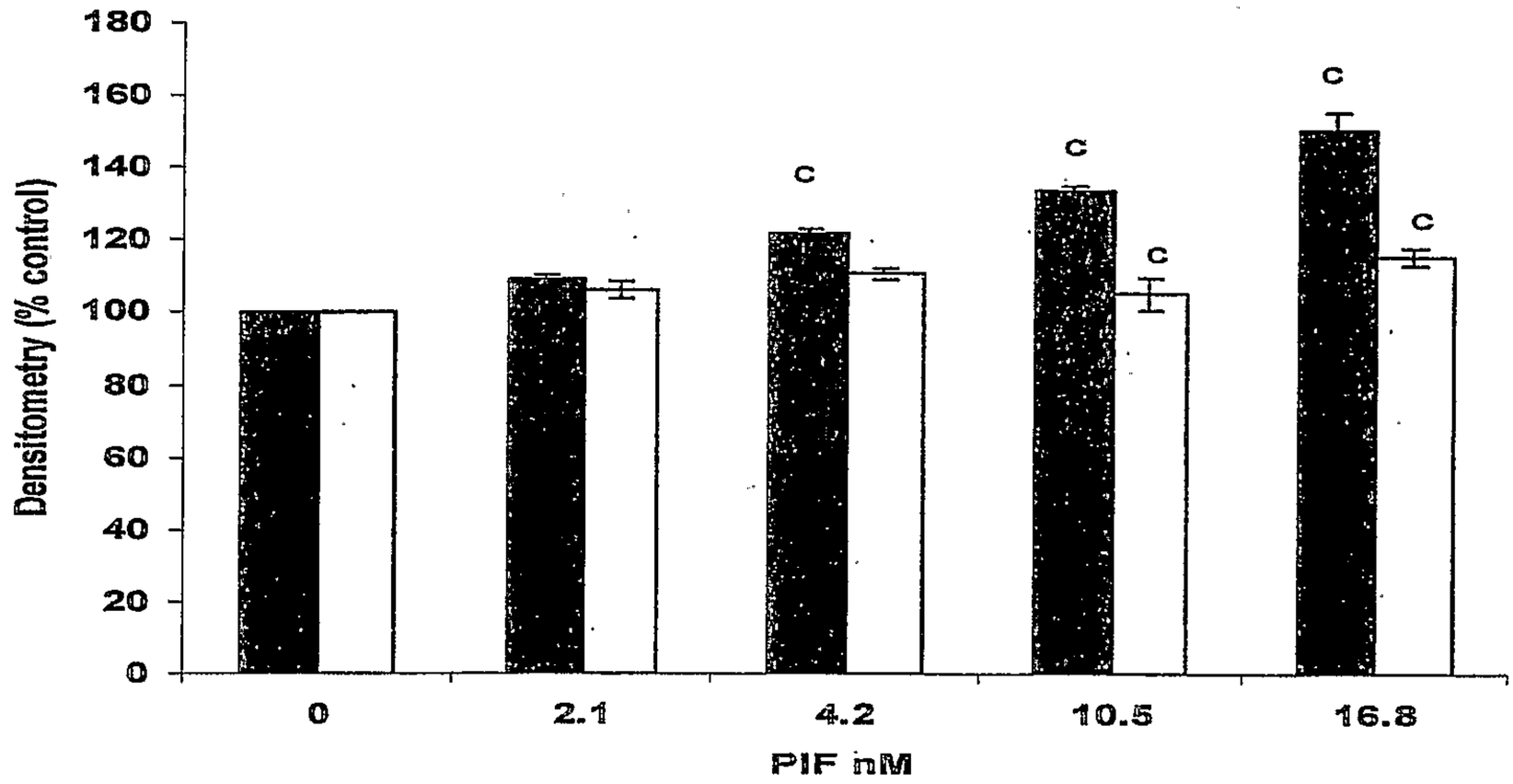
Figure 12

Figure 13

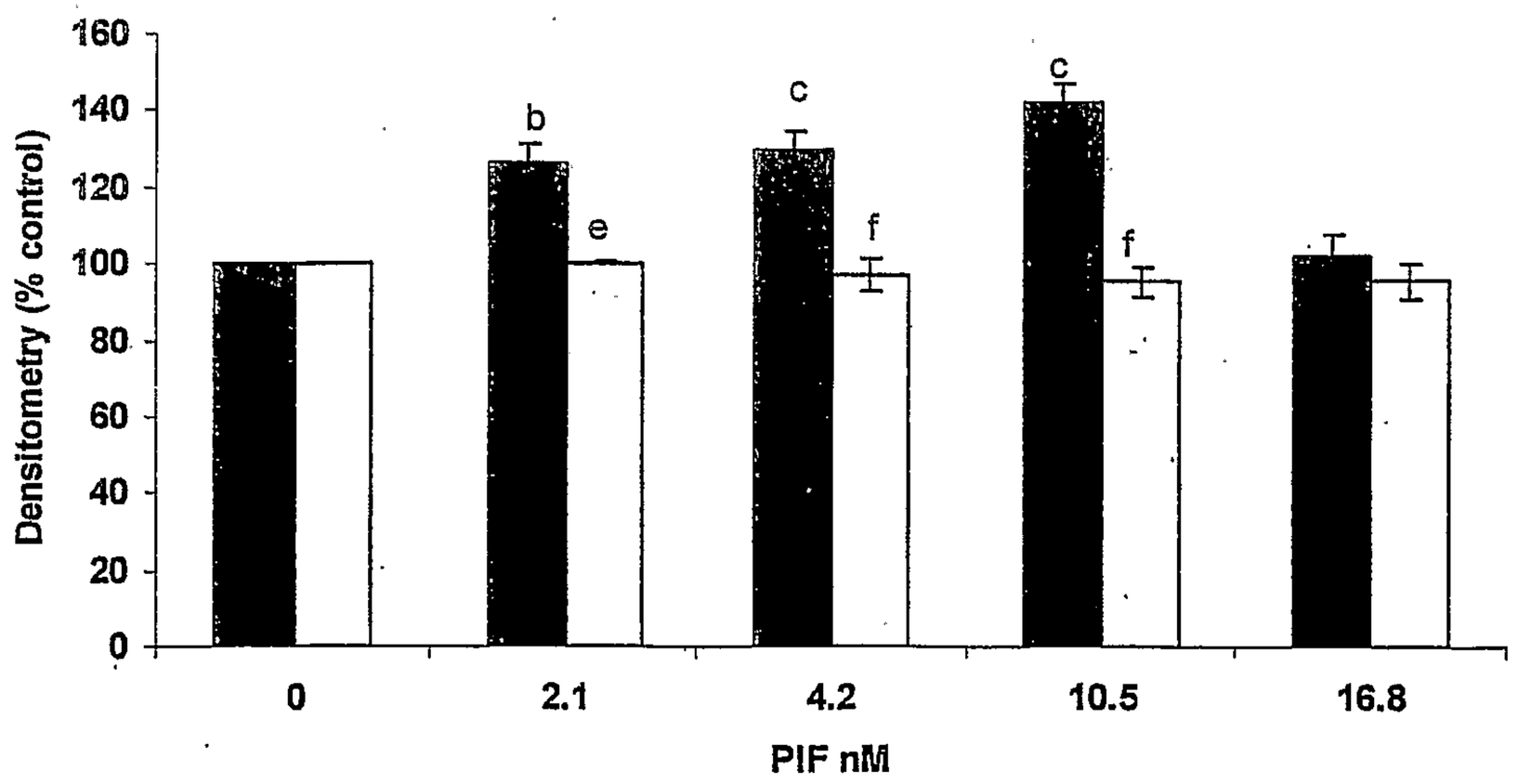
A



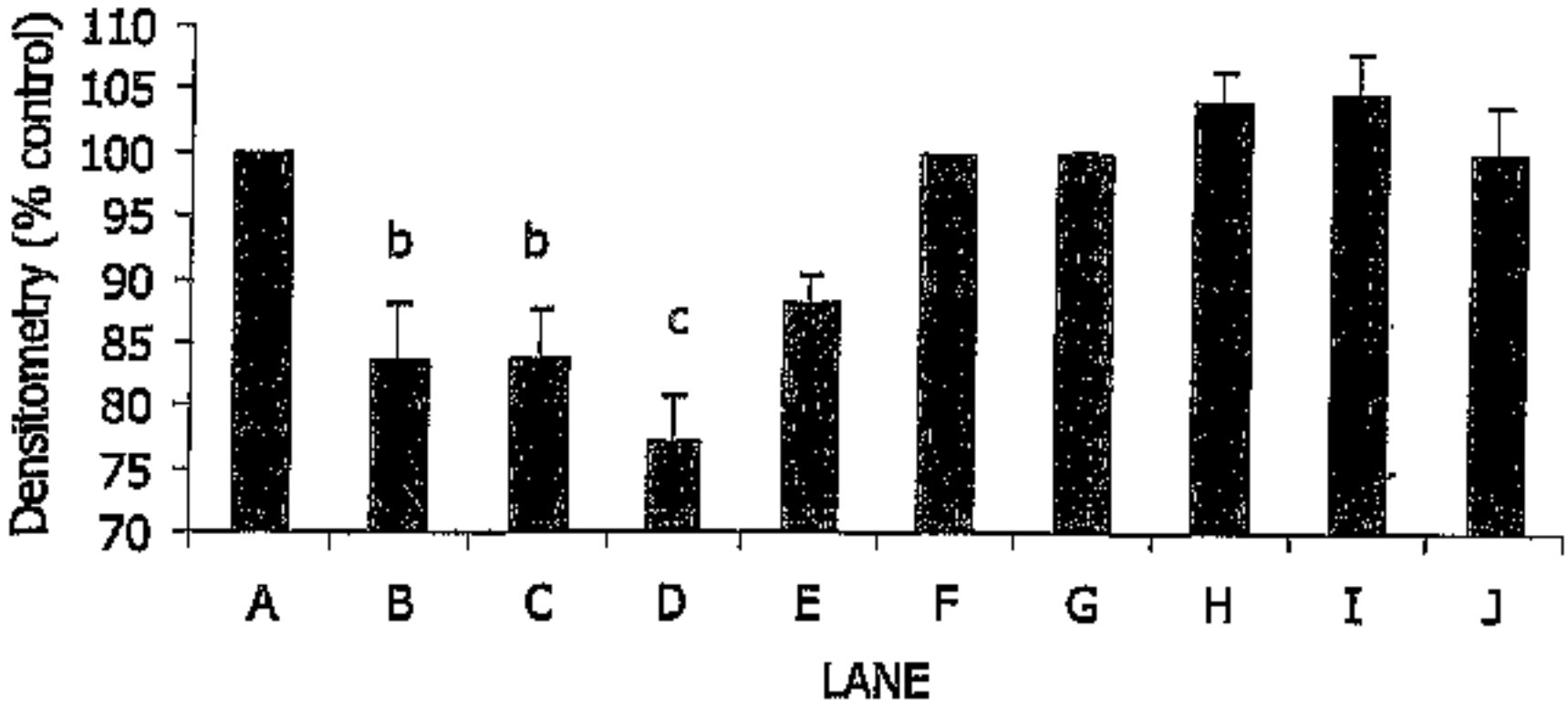
B



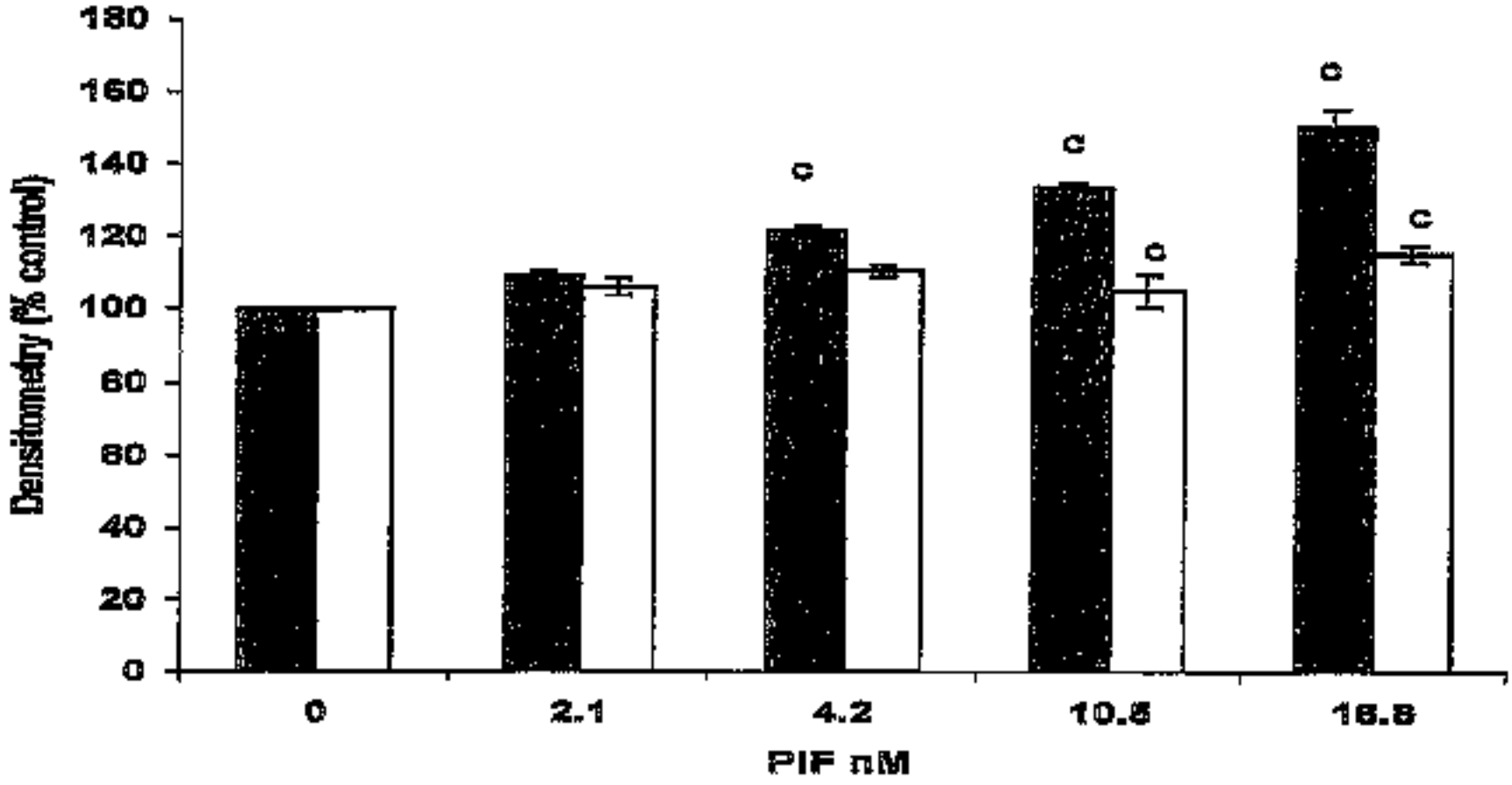
C



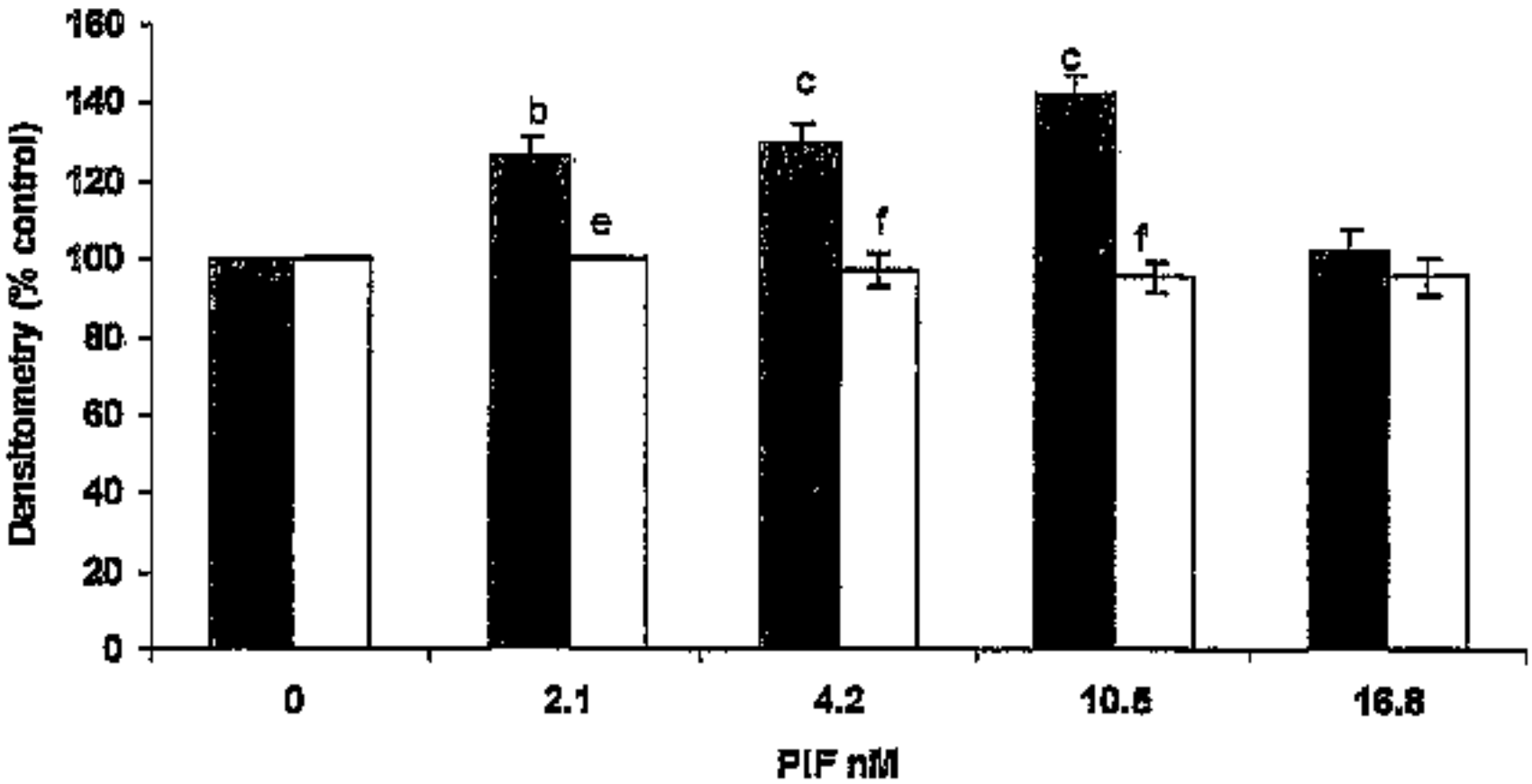
A


Densitometry (% control)
110
105
100
95
90
85
80
75
70
b
b
c
A
B
C
D
E
F
G
H
I
J
LANE

B


Densitometry (% control)
180
160
140
120
100
80
60
40
20
0
c
c
c
c
0
2.1
4.2
10.5
16.8
PIF nM

C


Densitometry (% control)
160
140
120
100
80
60
40
20
0
b
e
c
f
c
f
0
2.1
4.2
10.5
16.8
PIF nM