Title: METHOD OF PROCESSING MILK

Abstract: The present invention is directed to the use of lipoprotein lipase (LPL) in the prevention and intervention of metabolic syndrome and seborrhea and their pathologies, and processes for the preparation of medicaments containing bioactive, stabilized LPL for use by humans. The present invention is also directed to a method for the medical oral use of LPL. The present invention is further directed to nutraceutical and pharmaceutical compositions which contain active and/or stabilized milk LPL, processes for preparation of such nutraceutical and pharmaceutical compositions, and methods for use of such nutraceutical and pharmaceutical compositions.
METHOD OF PROCESSING MILK

The present invention claims priority on United States Provisional Patent Application Serial Nos. 60/958,741 filed July 9, 2007 and 61/074,445 filed June 20, 2008, both of which are fully incorporated herein by reference.

The present invention is directed to the use of lipoprotein lipase (LPL) in the prevention and intervention of metabolic syndrome and seborrhea and their pathogomies, and processes for the preparation of medicaments containing bioactive, stabilized LPL for use by humans. The present invention is also directed to a method for the medical oral use of LPL. The present invention is further directed to nutraceutical and pharmaceutical compositions which contain active and/or stabilized milk LPL, processes for preparation of such nutraceutical and pharmaceutical compositions, and methods for use of such nutraceutical and pharmaceutical compositions. The present invention is also directed to foods and/or a dietary supplement that contain biologically active, thermostable laetateal LPL, which can be useful for the treatment of certain diseases and/or conditions.

BACKGROUND OF THE INVENTION

One difference between milk and many other animal products is that milk is often consumed raw and, therefore, its edibility closely depends on the condition of the animal it comes from. In other words, the bacteria or viruses an animal carries, will often times pass into the milk. Pasteurization was invented in 1862 as a way to minimally cook a heat-sensitive food, namely wine, in order to destroy spoilers in it while preserving its character. In 1886, it was hypothesized that pasteurization could work for milk - that it could preserve its natural qualities and prolong its shelf life, while, more importantly, also destroying any pathogens it might contain. At such time, informational milk cultures were not available. Society, initially skeptical, was eventually persuaded to try out pasteurized milk.

A decline in milk-related illnesses was seen, immediately, but less noticed were journalized studies that reported a positive salutary superiority of raw milk over pasteurized milk. This problem was later found to be, in part, addressable by requiring pasteurized milk to be fortified with some vitamins and minerals that were impaired by the process of pasteurization; but, as for further documented inferiorities of milk that has been pasteurized to its raw counterpart and its apparent loss of certain curative properties, no research has ever been
conducted. As such, comparative results for long-term studies are unavailable.

According to prevailing scientific opinion, though, milk's loss of integrity due to pasteurization, beyond what is thought to be more than made up for by fortification, is impossible, because, basically, the only indigenous content not accounted for in conventional milk post-pasteurization are the enzymes that undergo denaturation. However, these enzymes only serve to spoil the milk and, so, are not desirable anyway. The heat of pasteurization inactivates LPL, an enzyme found naturally in milk.

The basis of the present invention was the search by the inventor for a safe and effective means to reduce sebum production in order to treat acne. Such a means, as discovered, involves the selective reduction of plasma lipid levels by the administration of LPL.

In the native state, LPL is an approximately 100-kDa, homodimeric glycoprotein. The two subunits are noncovalently bound, head-to-tail. The dimer is unstable by itself and tends toward dissociation into monomers. Full denaturation is reversible, in the presence of calcium. Active LPL functions as a rate-limiting glycerol ester hydrolase and, whether inactive or not, as a ligand for the cellular retention of select plasma lipids as for local lipolysis or endocytosis. The pH limits of LPL activity are 6 and 10; and the pH and temperature optima are 8-9 and 37° C, respectively. At optimum conditions, the half-life of LPL is about 2 hours. Higher temperatures and pHs speed up the loss of activity, but refrigeration slows it down; freezing, more so; and lyophilization preserves it very well.

LPL is divided into two source-types: animal and microbial. Microbial LPL is popular to work with in labs because it is easily produced and purified. Animal LPL may be derived from various animal tissues, blood, and mammalian milk. Fresh raw bovine milk contains about 1-2 mg/L of LPL.

LPL is a major clearing factor for plasma lipids. In 1993, after synthesizing and testing 100 different drugs, NO-1886 (ibrolipim) was identified as the best activator of LPL-activity and was proposed for the treatment of hyperlipidemia and related conditions. At such time, hyperlipidemia was also being established as a condition closely related to metabolic syndrome. In 1994, the National Cholesterol Education Program (NCEP), in their second report by expert panel on the detection, evaluation, and treatment of high blood cholesterol in adults (ATP II), associated obesity with hyperlipidemia, naming it as a major risk factor for ASCVD. In 1995,
studies found LPL underexpression to be an exacerbating factor in obesity and hyperinsulinemia. In 1998, the LPL-activator drug, NO-1886, was shown to be effective in treating insulin resistance and associated dyslipidemia in rats; and, in 2000, the same drug was shown effective in the treatment of obesity in rats. In 2001, ATP III, introduced a section on Metabolic Syndrome, wherein the pathology was proposed to be a collection of risk factors for ASCVD that associate with obesity. Further lab tests of NO-1886 bolstered the connection between diabetes, hyperlipidemia, and Metabolic Syndrome and added to the increasing evidence that LPL was an effective therapy for all these pathologies and related pathomeries.

In 1994, gemfibrozil’s efficacy was found to be associated with an increase LPL activity. In 1996, another test confirmed that the lipid-lowering effect of fibrates was associated with an enhancement of LPL activity. In 1999, tests showed that both fibrates and statins increased LPL activity. Simvastatin, in particular, increased LPL activity by 70%. In 2004, based on a systematic review of all the literature, the American College of Physicians (ACP) published guidelines for the treatment of type two diabetes and associated vascular complications. The recommendations stated that statins should be the primary prevention against vascular complications in at-risk patients and that at least moderate use of statins should be considered for the improvement of patients not at risk. In 2005, a test of pravastatin, simvastatin, atorvastain, and pitavastatin showed that they all increase LPL activity. Pitavastatin was found to increase LPL activity the most.

In 2007, AMT-011, a patented LPL-gene replacement therapy, obtained an orphan drug designation in both Europe and the US for the treatment of LPL-deficiency disorders. In 2008, the inventors of AMT-011 were awarded a patent by the EPO for its use in treatment of NAFLD, NASH, and metabolic syndrome.

The scientific consensus appears to be that LPL therapy could be an effective means of prevention and intervention for hyperlipidemia, diabetes, and the wider condition and pre-condition of metabolic syndrome.

A relevant distinction is often made between the modes of expression of LPL. After transcription in parenchymal cells, LPL is transported by transcytosis to the luminal surface of the capillary endothelium. Thence, LPL extends into the plasma and is involved in the binding, lipolysis, and uptake of lipids. Local eLPL activity and availability is modulated by degrees of
cellular internalization and deactivation, by reverse transport to the parenchyma, and by product inhibition. Negative feed-back by FFA is also an important mechanism whereby LPL-lipoprotein complexes are dislodged from the endothelium in order to stop local lipolysis. As aside from inhibitory reasons, it has been shown that LPL is continuously released into the plasma from the endothelium under physiologic conditions and that an equilibrium is maintained between eLPL expression and parenchymal production. Endogenous pLPL is largely inactive (>95%); although, while inactive, LPL still contributes to lipolytic efficiency and lipid uptake by forming complexes with lipids that better bind to cells. Dislodgement is also a means of regulatory tissue-to-tissue redistribution, as from adipose to muscle when exercising or vice versa after feeding. PLPL has a much lower \( K_m^{\text{app}} \) (0.20 ± 0.03 mM) and higher corrected \( V_{\text{max}}^{\text{app}} \) (11.4 ± 0.1 mM) than eLPL (0.36 ± 0.11 mM and 1.2 ± 0.1 mM, respectively); thus, when active, it is a far superior catalyst. It has been proposed that the release of active eLPL may be essential for efficient lipolysis. PLPL mass, irrespective of activity, has also been shown to be positively correlated and more advantageous than actionable eLPL with regard to the clearance of plasma lipids and insulin sensitivity. PLPL is subject to rebinding to the endothelium, as in adipose or muscle tissues, which also has a stabilizing effect on the LPL, or to uptake and degradation by the liver.

NO-1886, AMT-011, and other lipid-lowering drugs that upregulate LPL expression all work at the level of transcription to indirectly express eLPL; therefore, they all rely on a non-defective LPL-pathway to the endothelium for maximum efficiency. LPL-replacement therapy, which would introduce pLPL into circulation, could be supremely effective, except that a method for such treatment has not yet been invented that makes it a feasible option. Pure, non-toxic animal LPL, fit for human use, is costly to harvest, preserve, and prepare for administration. Therefore, the natural turnover rate of LPL has made its therapeutic replacement too risky to explore. For instance, when exogenous LPL is injected intravenously, it may persist in circulation as an active dimer, then as monomers after it loses its activity; or be bound by the endothelium, as in adipose or muscle tissue, which would lengthen its half-life. At sometime in the prosecution of its clearing work, LPL will be degraded by the liver, probably as a LPL-lipid-complex. This happen minutes after its injection or after the LPL has been bound and released by the endothelium numerous times before it is finally caught by the liver. Therefore, replacement therapy might have to consist of repeated dosage administrations throughout the day
and especially after meals. The half-life of pLPL has been variously estimated in experiments to be 10-40 min; and the half-life of eLPL to be about 2 hours. After an hour, about 10% of an injected sample of LPL may be found actionably exposed on the endothelium. Because LPL is saturable, its half-life and persistence should increase with plasma lipid concentrations.

**SUMMARY OF THE INVENTION**

The present invention is directed to the use of LPL in the prevention and intervention of metabolic syndrome and seborrhea and their pathomgies, and processes for the preparation of medicaments containing bioactive, stabilized LPL for use by humans. The present invention is also directed to a method for the medical oral use of LPL. The present invention is further directed to nutraceutical and pharmaceutical compositions which contain active and/or stabilized milk LPL, processes for preparation of such nutraceutical and pharmaceutical composition, and methods for use of such nutraceutical and pharmaceutical compositions. The present invention is also directed to foods and/or a dietary supplement that contain biologically active, thermostable milk LPL, which can be useful for the treatment of certain pathologies. The present invention is directed to a method for the prophylactic and therapeutic use of lipoprotein lipase, comprising oral administration. The present invention is also directed to nutraceutical and pharmaceutical compositions which contain biologically active, stabilized milk lipoprotein lipase for the treatment of metabolic syndrome and seborrhea and their pathomgies (e.g., skim milk, milk containing non-homogenized milk fat, casein isolate, milk soluble protein isolate, soluble casein isolate; and powderize and pilled compositions thereof). The present invention is also directed to methods for the use of the nutraceutical and pharmaceutical compositions (e.g., oral administration, etc.).

Key Abbreviations used in this invention are as follows: lipoprotein lipase (LPL); endothelial LPL (eLPL); plasma LPL (pLPL); triglyceride (TG); free fatty acid (FFA); apparent maximum reaction velocity (V_{max app}); apparent Michaelis-Menten constant (K_{m app}); high density lipoprotein (HDL); low density lipoprotein (LDL); intermediate density lipoprotein (IDL); triglyceride-rich lipoprotein (TRL); very low density lipoprotein (VLDL); chylomicron (CH); non-alcoholic fatty liver disease (NFLD); peroxisome proliferator-activated receptor (PPAR); sebum excretion rate (SER); natural moisturizing factor (NMF); milkfat globule membrane (MFGM); micellar calcium phosphate (MCP).
Definitions of several terms used in the invention are as follows:

**Hyperseborrhea**: absolute overproduction of sebum.

**Metabolic syndrome**: the homeostatic imbalance of glucose and/or lipid metabolism marked by the presentation of one or more of a constellation of indications that may be ameliorated by lipoprotein lipase therapy.

**Nutraceutical**: a nutritive medicament or, at least, one that is generally recognized as safe.

**Nutracoology**: the prophylactic and therapeutic properties of a nutraceutical.

**Seborrhea**: a medical, cosmetic, or hygienic condition related to the secretion of sebum.

In summary, the present invention is directed to the use of edible lipoprotein lipase for the intervention and prevention of metabolic syndrome and seborrhea and their pathomogies.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition for the treatment of metabolic syndrome and seborrhea and their pathomogies, comprising milk or one of its fractions or derivatives, where said composition contains active and stabilized lipoprotein lipase.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition for the treatment of metabolic syndrome and seborrhea and their pathomogies, comprising a skim milk that contains active and stabilized lipoprotein lipase.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathomogies that is made into a powder, as by evaporation, spray-drying, and/or freeze-drying.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathomogies that is recombined in some proportion with non-homogenized milkfat.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathomogies that is processed to make a milk soluble protein isolate.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome...
and seborrhea and their pathogomies that is made into a pill with a suitable carrier.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathogomies that is processed to make a casein isolate.

The present invention is also or alternatively directed to a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathogomies that is processed to make a soluble casein isolate.

The present invention is also or alternatively directed to process for the preparation of a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathogomies from milk (e.g., skim milk, milk containing non-homogenized milk fat, milk soluble protein isolate, casein isolate, soluble casein isolate, etc.) which includes the steps of: a) warm separation of the cream (e.g., up to 41 °C); b) cooling of the milk (e.g., down to 0 °C), c) homogenization of the cooled milk, along with the optional mixing in of known, suitable buffering and/or stabilizing agents to the cooled milk until the LPL is thermostable.

The present invention is also or alternatively directed to process for the preparation of a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL for the treatment of metabolic syndrome and seborrhea and their pathogomies from milk (e.g., skim milk, milk containing non-homogenized milk fat, milk soluble protein isolate, casein isolate, etc.) which includes the steps of: a) warm separation of the cream (e.g., up to 41 °C); b) cooling of the milk (e.g., down to 0 °C), c) homogenization of the cooled milk, along with the optional mixing in of known, suitable buffering and/or stabilizing agents to the cooled milk until the LPL is thermostable, and d) recovering soluble casein.

The present invention is also or alternatively directed to process for the preparation of milk soluble protein isolate which includes the steps of: a) solubilization of the micellar calcium phosphate from a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL by the mixing in of a chelating agent (e.g., sodium carbonate and/or sodium citrate, etc.) in a select concentration at given temperature (e.g., 0-25 °C, etc.) and/or for a given period of time (0.1-30 minutes, etc.), wherein no more or essentially no more κ and β-casein can be solubilized, and to which temperature the nutraceutical or pharmaceutical composition that
contains active and/or stabilized LPL is also brought - in order to chelate the calcium, whereupon the chelate complexes can be optionally removed, b) agitating the nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL at a low temperature (e.g., less than 20° C, 0° C, etc.) such by, but not limited to, homogenization, c) precipitating casein micelles by rennet at a low temperature (e.g., 0-25° C), d) removing the precipitate, and the trapped water therein is thoroughly extracted and put back into the aqueous phase.

The present invention is also or alternatively directed to process for the preparation of casein isolate which includes the steps of: a) mixing calcium into a nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL at a given temperature wherein no more or essentially no more κ and β-casein can be insolubilized at a given temperature (e.g., 30-50°C, 41°C, etc.), to which temperature the nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL is also brought, and b) precipitating casein micelles.

The present invention is also or alternatively directed to process for the preparation of casein isolate which includes the steps of: a) dispersing casein isolate in demineralized water at or near a concentration wherein the highest amount of κ and β-casein can be finally solubilized (e.g., 1-25%, 5%, 10%, 5%, etc.), b) solubilizing the micellar calcium phosphate from the dispersion by mixing in of a chelating agent (e.g., sodium carbonate and/or sodium citrate, etc.) at a given concentration and at a given temperature down to 0° C., where no more or almost no more κ and b-casein can be solubilized, to which the temperature the casein isolate is also brought - in order to chelate the calcium, whereupon the chelate complexes can be optionally removed, c) thoroughly breaking up the dispersed composition by agitating (e.g., high pressure homogenization, etc.), and d) separating the aqueous phase from the insoluble phase (e.g., centrifugation, filtration, etc.).

The present invention is also or alternatively directed to a method for the intervention or prevention of metabolic syndrome and seborrhea and their pathogemies comprising the oral administration of nutraceutical or pharmaceutical composition that contains active and/or stabilized LPL in a dosage more or less equal to the USDA-recommended serving or one that is efficacious.

The present invention is also or alternatively directed to a food useful for the intervention
and prevention of metabolic syndrome and seborrhea and their pathogomies, comprising skim milk with active, thermostable LPL.

The present invention is also or alternatively directed to a food useful for the prevention of metabolic syndrome and seborrhea and their pathogomies, comprising standard whole milk and/or skim milk with active and thermostable LPL (e.g., BIOGALACT™).

The present invention is also or alternatively directed to a dietary supplement useful for the intervention and prevention of metabolic syndrome and seborrhea and their pathogomies, comprising a protein isolate form of milk and/or skim milk with active and thermostable LPL (e.g., BIOGALACT™).

The present invention is also or alternatively directed to a supplement that is made into a powdered form.

The present invention is also or alternatively directed to a supplement that is encapsulated in a dosage more or less equal to the amount in the recommended serving of milk or one that is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of the metabolic syndrome and seborrhea and their pathogomies comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ in a daily dosage more or less equal to the amount in the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of xanthomatosis comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of atherosclerosis comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of seborrhea and its pathogomies comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ a daily amount more or less equal
to the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of acne comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a method for the intervention or prevention of eczema or psoriasis comprising the oral administration of a supplement that includes active, thermostable LPL and/or BIOGALACT™ a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

The present invention is also or alternatively directed to a process for the production of the food that includes active, thermostable LPL and/or BIOGALACT™ comprising the steps of a) separating raw whole milk to form the skim milk fraction, said step of separating including the use of separator, microfilter or combinations thereof, b) optionally homogenizing of the skim milk fraction along with the optional addition of lipophilic vitamins or other stabilizers, until the LPL is at least partially thermostable, and c) optionally pasteurizing the homogenized skim milk fraction.

The present invention is also or alternatively directed to a process for the production of the food that includes active, thermostable LPL and/or BIOGALACT™ comprising the steps of a) separating raw whole milk to form a cream fraction and a skim milk fraction, b) homogenizing of the skim milk fraction along with the optional addition of lipophilic vitamins or other stabilizers, until the LPL is at least partially thermostable, c) optionally homogenizing the cream fraction, d) recombining the cream fraction and the skim milk fraction, and e) optionally pasteurizing the recombined cream fraction and skim milk fraction.

The present invention is also or alternatively directed to a process for the production of the food that includes active, thermostable LPL and/or BIOGALACT™ comprising the steps of a) separating raw whole milk to form the skim milk fraction, said step of separating including the use of separator, microfilter or combinations thereof, b) optionally homogenizing of the skim milk fraction along with the optional addition of lipophilic vitamins or other stabilizers, until the LPL is at least partially thermostable, c) optionally pasteurizing the homogenized skim milk fraction, and d) ultrafiltrating said skim milk fraction with diafiltration for the milk protein.
retentate.

The present invention is also or alternatively directed to a process for the production of the food that includes active, thermostable LPL and/or BIOGALACT™ comprising the steps of a) separating raw whole milk to form a cream fraction and a skim milk fraction, b) optionally homogenizing of the skim milk fraction along with the optional addition of lipophilic vitamins or other stabilizers, until the LPL is at least partially thermostable, c) optionally homogenizing the cream fraction, d) optionally recombining the cream fraction and the skim milk fraction, e) optionally pasteurizing the skim milk fraction or recombined cream fraction and skim milk fraction, and f) ultrafiltrating said skim milk or recombined cream fraction and skim milk fraction with diafiltration for the milk protein retentate.

The present invention, in an envisioned preferred embodiment, comprises duel clarification and separation of fresh, raw milk; partial homogenization of the skim milk, where known stabilizers of LPL are added; followed by standardization; pasteurization; and packaging. Partial pasteurization of both parts may also precede standardization.

The present invention is also or alternatively comprises a milk soluble protein (MSP) isolate prepared from the skim milk of the present invention, where the micellar calcium phosphate (MCP) of said milk is solubilized, as by the chelation of the calcium; and the chelate complexes may be subsequently removed, as by skimming, straining, or filtration. The chelating agent may be any non-toxic substance such as a sodium carbonate or sodium citrate. The chelator is added in the critical concentration where no more k and b-casein may be dissociated from micelles at a given temperature (e.g., 0-25 °C, etc.), for a given incubation period (e.g., 40-80 millimoles per liter of trisodium citrate at 25-35 °C for 1-10 minute, etc.), to which target temperature the skim milk is also brought and closely maintained at: the lower the temperature the more soluble the caseins become and the less calcium needed to induce complete solubilization. Then, the skim milk is agitated, as by homogenization, so as to speed up the solubilization process, but at a temperature not rising substantially above the target maximum temperature of the prior step, so as not to allow any caseins to substantially re-insolubilize or salt back out. Then, the casein micelles are precipitated by rennet - also at the target temperature - and the precipitate is removed; and the entrapped water is extracted back into the aqueous phase. The water-soluble proteins of the aqueous phase may then be spray-dried and encapsulated.
The present invention is also or alternatively comprises a casein isolate prepared from the starter skim milk of the present invention by any known process for doing so, where the improvement comprises the addition of calcium to said milk in the critical concentration where no more κ and β casein may be associated with the micelles at a given temperature (e.g., 75-200 milimoles per liter of calcium chloride at 30-45°C, etc.), to which target minimum temperature the skim milk is also brought; then rennet is mixed in to precipitate the casein micelles, at a temperature not falling substantially below the target minimum temperature, so as not to allow a substantial solubilization of caseins; the precipitate is removed, which may then be spray-dried and encapsulated.

The present invention is also or alternatively comprises a soluble casein isolate prepared from the casein isolate of the present invention described above, where said precipitate is dispersed in demineralized water at a concentration of 1 to 25% or the optimum concentration where the highest amount of κ and β-casein may be finally solubilized; the MCP of the dispersion is removed as by chelation, as described above; and the coagulated casein is broken up and the dispersion is agitated, as by high pressure homogenization (HPH) - so as to promote and speed up the dissociation and solubilization of the more readily solubilized caseins - and where the target maximum temperature is not substantially exceeded. Then, the aqueous phase is separated from the insoluble casein, as by centrifugation or filtration, also at a temperature not substantially exceeding the target maximum. The solution may then be spray-dried and encapsulated.

These and other aspects and advantages will become apparent to those skilled in the art upon reading and following the description.

**DETAILED DESCRIPTION OF THE INVENTION**

The present invention is directed to the use of a convenient and/or inexpensive LPL therapy to prevent and/or improve the primary and/or secondary conditions of metabolic syndrome, hyperlipidemia, and/or diabetes.

Purified LPL is commercially available and is mainly used for research purposes. It is derived from Pseudomonas species or milk. Current methods of isolation of LPL from milk mostly involve heparin affinity chromatography; and while they are able to render a highly pure product, they do not generally produce one fit for human consumption. A high degree of purity,
however, is not absolutely necessary for the efficacy of the present invention. In fact, since, of mostly the constituents of milk, LPL has its strongest affinity towards the albumin and casein, the mere processing of the milk through a standard separator could sufficiently purify the LPL for use in all of the present methods of treatment. Furthermore, it is believed that LPL is so potent and in such proportions in bovine milk that one need not even separate it from the milk fat to see results; that is to say, whole milk may also be drunk to derive a net nutracologic benefit from the LPL.

Fresh raw milk contains active LPL, but the activity is highly unstable and rapidly diminishes after milking from an animal. Refrigeration slows down the loss of activity and lyophilization preserves it very well. However, LPL's activity is almost totally destroyed by pasteurization. The present invention involves a novel process for the preservation of the activity of milk LPL that is specific, ingenuous, easy, inexpensive.

As set forth above, the present invention involves the use of a convenient and inexpensive LPL therapy to prevent and improve the primary and secondary conditions of metabolic syndrome, hyperlipidemia, and diabetes. Metabolic syndrome is a breakdown of the homeostasis of glucolipid metabolism. The indications are numerous and vary by case, but primarily include: diabetes, hyperlipidemia, obesity, abdominal adiposity, hepatic steatosis, renal disease, and pancreatitis. Currently, the safest and most effective treatment for metabolic syndrome is insulin-replacement therapy. However, this actually causes hypoglycemia and, naturally, over time, exacerbates insulin resistance. Therefore, insulin-replacement therapy is used only as a last resort. Glucose and lipid metabolism are closely related. Specifically, endogenous triglyceride (TG) is manufactured from glucose in the liver and hepatic gluconeogenesis mainly refers to the hydrolysis of TG to produce glucose. Endogenous TG is the primary constituent of VLDL which is also synthesized in the liver; and the primary clearing factor for VLDL is LPL, which is upregulated by insulin - but the primary mediator for the hepatic uptake of plasma lipids, including VLDL, is hepatic lipase (HL), which is downregulated by insulin. Insulin is upregulated by blood glucose and is its primary clearing factor.

Among the first predictors of metabolic syndrome is an LPL-deficient lipid profile or dyslipidemia, which is characterized by high TRL and low HDL levels. In particular, VLDL-emia has been shown to have a strong correlation with the accumulation of fat in the liver and
high blood glucose levels. A theoretical pathogenesis from here starts with the increased contribution of VLDL components into the liver by the mediation of HL; which adds to fat stores and stimulates gluconeogenesis, as for de novo VLDL production, since VLDL uptake indicates its removal from plasma. But, if the VLDL level in the blood is high, a negative feed-back mechanism diverts some glucose from the VLDL-production pathway, which contributes to an increase in blood-glucose levels. Glucose stimulates the secretion of insulin, which, in turn upregulates LPL expression and glucose uptake, as for postprandial clearance. But insulin exposure causes desensitization to insulin. Therefore, a persistently high level of VLDL at the outset, via persistent hyperglycemia and hyperinsulinemia, leads to insulin resistance - if not caused by it. As such, a vicious cycle is created. Hence, systemic lipid uptake is increased due to the larger lipid pool and specific insulin resistance, which may cause secondary complications in the pancreas, kidneys, adipose tissue (obesity), skin (xanthomatosis), arterial endothelium (atherosclerosis) and, especially, the liver, which may also have acquired insulin-resistant HL. Therefore, secondary hepatosteatosis, hypertriglyceridemia, hyperglycemia, hyperinsulinemia and the wider primary condition of Metabolic Syndrome in many cases may not start with hepatic insulin resistance, as much, as it does with LPL insulin resistance. This is not to say that LPL deficiency would necessarily lead to VLDL-emia, et seq. For the clearance of VLDL is not nearly as dependent on LPL, as is that of CH, for instance. Thus, in LPL deficiency disorders, other pathways of VLDL clearance may suffice to normalize VLDL levels. Such alternate pathways may even be augmented in congenital LPL deficiency, as are LPL-independent pathways for adipose lipid accumulation in familial LPL deficiency. Nevertheless, among the family of plasma lipases - which consists of LPL, HL, and endothelial lipase (EL) - only LPL prefers VLDL as a substrate; and, hence, it is its primary catabolizer. Therefore, while an absolute underexpression of LPL will not always equate with VLDL-emia et seq., an increase of fully functional LPL could be a highly effective means of decreasing VLDL levels. Accordingly, the constellation of indications of metabolic syndrome may be linked by their susceptibility to LPL treatment. Hence, LPL-replacement therapy could be an effective means of intervention and prevention for metabolic syndrome.

Hypercholesterolemia, which is included in the hyperlipidemias as type II and III, is not always included in metabolic syndrome; but, with respect to the present invention it is, since it
is indicated by abnormal lipid metabolism and an increase in LPL expression may be an effective means of treating it.

**Fredrickson Classification of the Hyperlipidemias**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Lipoproteinemia</th>
<th>Atherogenicity</th>
<th>Relative Frequency, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CH</td>
<td>None</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IIa</td>
<td>LDL</td>
<td>High</td>
<td>10</td>
</tr>
<tr>
<td>IIb</td>
<td>LDL &amp; VLDL</td>
<td>High</td>
<td>40</td>
</tr>
<tr>
<td>III</td>
<td>IDL</td>
<td>High</td>
<td>&lt;1</td>
</tr>
<tr>
<td>IV</td>
<td>VLDL</td>
<td>Low</td>
<td>45</td>
</tr>
<tr>
<td>V</td>
<td>VLDL &amp; CH</td>
<td>Low</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type</th>
<th>Lipoproteinemia</th>
<th>Atherogenicity</th>
<th>Relative Frequency, %</th>
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</thead>
<tbody>
<tr>
<td>Hypercholesterolemia</td>
<td>LDL, IDL, &amp; VLDL</td>
<td>High</td>
<td>50%</td>
</tr>
<tr>
<td>Hypertriglyceridemia</td>
<td>CH &amp; VLDL</td>
<td>Low</td>
<td>50%</td>
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</table>

Hypercholesterolemia is marked by elevated LDL, IDL, and/or VLDL, which does not indicate absolute LPL underexpression, since LDL and IDL are byproducts of LPL-mediated hydrolysis of TRL. In fact, familial cholesterolemia is due to a genetic defect of the LDL receptor. In any case, the functions of LPL are well-suited to improving the condition. LPL is a glycerol ester hydrolase and a ligand of lipids for the promotion of their cellular lipolysis and/or uptake. It has also been proposed that common heterozygous mutations in the LPL gene resulting in relative deficiency may be an underlying cofactor in many incidences of CVD.

In particular, an increase of LPL could afford treatment by intensifying the lipolysis of LDL and IDL and creating more pathways for the endocytosis of LDL and IDL and their precursors, including non-receptor dependant ones.

Furthermore, LPL contributes to the synthesis, enhancement, and half-life of HDL, which, in itself, is a major factor in the clearance of blood cholesterol. Nascent HDL is generated primarily by the liver or my derive from the LPL-mediated hydrolysis of TRL. Lipolysis of TRL releases apolipoproteins and phospholipids that are required for HDL augmentation and maturation. HDL is depleted by the CETP-mediated exchange with other lipoproteins of cholesterol esters for TG and subsequent hydrolysis by HL and EL. Thus, in the presence of high levels of TRL, HDL undergoes rapid delipidation; whereupon, as HDL₃, it is subject to removal from circulation. But a decrease in the TRL pool would downregulate HDL delipidation.
Therefore, an increase in LPL is conducive to an increased, enhanced, and prolonged presence of antiatherogenic HDL cholesterol in plasma.

In general, the antiatherosclerotic effect of LPL consists in the decrease of plasma lipid pools, which decreases the mathematical chance of vascular injury during lipid uptake and, hence, the chance that mobilized macrophages might malfunction and start accumulating lipids at the site. And, additionally, in the increase of the turnover rate for atherogenic lipoproteins, which decreases their time in serum and, hence, decreases the extent to which they may undergo oxidation. Oxidized lipoproteins are more liable to get stuck in the vascular wall, in the first place, and more prone to actually cause macrophagic malfunction. Science and literature also support the use of LPL-replacement therapies to treat pathologies that are the direct or indirect result of the production of sebum. So far, the most effective way to reduce sebum production is with isotretinoin, which is a category X teratogen.

Sebaceous glands attach to ducts, which several may share, and are found everywhere on the skin, except the palms and soles. The glands excrete a holocrine product up the ducts called sebum. The sebaceous excretion is generated by the migration of differentiating sebocytes from the periphery of the gland into the duct. Sebocyte differentiation is characterized by lipid accumulation; and sebum is composed almost entirely of lipidic material. Sebum exudes onto the skin out of a pore that consist of the sebaceous duct by itself or in connection with a local follicle.

The pathogenesis of a wide range of skin conditions begins with the production of sebum. Theoretically, a seborrheic condition presents when there is an excessive sebum excretion rate (SER). In cases of absolute excess, oily skin is always indicated. Relative excess refers to cases where the SER is not extraordinary, although is greater than can be fluidly released through the sebaceous pores. This is due to an occlusion of the pores. External factors of occlusion include superficial decompression and the use of astringents. Spongiosis or intracutaneous edema also occludes pores by profound compression. But heat increases SERs (≈10%/1°C). Therefore, caloric or inflammatory spongiosis, as is caused by shaving, has an extra occlusive effect (although his may be more than made up for by the thermal decrease in viscosity of the sebum; nevertheless, shaving will cause spongiosis). Sebum may also oxidize and harden in the pore causing partial blockage; this is called an open comedo or blackhead. These are more likely to
occur in certain skin types and certain areas of the skin where pores are large and flexible and splay with the skin surface. Seborrhea in more sensitive and inflexible regions and skin types causes inflammation. When factors cause an occlusion such that the poric entry rate of sebum exceeds its exit rate, sebum builds up until the pore intracutaneously ruptures; whereupon, acantholysis by sebum and its continued accumulation give rise to a closed comedo or white head. If the oncotic factors are not promptly neutralized, a closed comedo will give rise to a marked spongiotic lesion; and, if not timely resolved, the comedo will abscess. Furthermore, if the steady state velocity and viscosity of sebum are high enough in relation to the size of lax pores, a persistent seborrheic condition will present. Depending on the skin type or poric character and the SER, seborrheic skin lesions will manifest themselves in one of the various forms of acne, eczema, or psoriasis. (Skin type, being an inherited trait, can show homogeneity by geographical region; therefore, so might seborrheic presentation. However, presentation is not dispositive of skin type, since SER and sebum composition factor into it and, thus different regions on one body may present differently; and, furthermore, poric character - in addition to, but independent of, sebogenesis - is hormone-sensitive, so, may vary by life cycle stage).

Sebaceous glycerol is a major natural moisturizing factor (NFM) of the skin. Glycerol, like other humectants, works by degrading the desmosomes that glue together corneocytes and thus promotes desquamation of the stratum corneum. Corneocytes are abnormally retained in xerosis. However, in high concentrations, glycerol has a keratolytic effect. Thus, glycerol, by desmosomal digestion, contributes to both intracutaneous acantholysis and supercutaneous exfoliation. A normal amount of sebum on the skin surface is essential for exfoliative hydration. But, in seborrheic conditions - where sebaceous glycerol and glycerol derived from the hydrolysis of sebaceous glycerides abound on the skin surface - exfoliative keratolysis occurs. Hence the condition commonly called combination skin (seborrheic xerosis), which is indicated by the skin appearing both oily and dry, at the same time. Thus, the severity and effect of seborrheic keratolysis is proportionate to the presence of surface sebum and dependent on the skin type. Scaling is the primary indication of seborrhea in veterinary medicine. On the scalp, seborrhea may cause dandruff and is indicated in certain patterns of hair loss. Seborrheic keratolysis is a common cause of various forms of pruritus and dermatitis.

Sebum contains several antioxidants, the most important of which is $\alpha$-tocopherol.
Redox is important because of the deleterious consequences of oxidation and especially so at surface of the skin which is an environment of high oxidative stress. Sebaceous squalene is susceptible to lipid peroxidation. This is a free radical reaction, catalyzed by UV radiation and ozone, that converts lipids into peroxides; and since squalene is also a prominent NMF, its oxidation may lead to both dermal desiccation and peroxide damage. Peroxide damage, like direct photo-oxidative damage, is a factor in premature aging and skin cancer. Therefore, redox for squalene is vital.

The hydrolysis of glyceride and the autoxidation of FFA are the two processes of rancidification. On excretion, sebum already contains a high concentration of FFAs; and most FAs in sebum are of the 16 and 18 carbon kinds, which are not intrinsically rancid. Therefore, FFA-autoxidation is the most important process in the rancidification of sebum. So, redox is also a factor in the regulation of certain body odors.

Now, α-tocopherol works by competitive oxidation. Therefore, since oxidation is also a chain-reaction, the redox effect of α-tocopherol is a logarithmic function of time. Thus, at the skin-air interface, a major factor in oxidative prevention should be the percutaneous resorption rate of sebum. However, in hyperseborrheic conditions, the stratum corneum is extremely saturated with sebum and, therefore, resorption is severely inhibited; so, oxidation is rampant. Hyperseborrhea, therefore, depending on the malodiferous content of the sebum and in proportion to sebum’s surface presence, will also cause sebaceous body odor.

Therefore, besides acne, eczema, and psoriasis, seborrhea may be associated with a number of other secondary conditions, including erythema, rosacea, xerosis, alopecia, spongiosis, dermatitis, pruritis, keratolysis, dandruff, premature aging, skin cancer, razor bumps, shininess, and body odor.

Frequent use of soaps or solvents is the most common way of controlling excessive sebum accumulation; however, this treatment removes most surface sebum, dries out the skin, and has an astringent effect. Therefore, this treatment regimen often exacerbates the problem.

Sebum production is a continuous process necessary for the health of the skin and hair and thus has a stable steady state value. The state variables are exogenous and endogenous lipids; that is, plasma lipids and lipids produced by de novo lipogenesis in the sebaceous gland, respectively. This means, however, as in most such weak homeostatic systems where the variables
are closely related, that the steady state value is adjustable up and down by resource stress. In other words, SERs will rise and fall with normal fluctuations in the plasma lipid pool until de novo lipogenesis can be recalibrated and homeostasis be reestablished. But, with abnormal dietary disruptions, SERs do not quite return to normal, whether coming off an increase or a decrease - that is, reactive compensation occurs and a new point of homeostasis is established. However, in practice, dietary modifications often do not produce clinically significant reductions in SERs. Specifically, low-fat diets often will not significantly improve seborrhea. This is due to the extrinsic upregulatory factor of LPL downregulation and redistribution by adipose tissue as plasma lipid levels decrease. Thus, during short term fasts, overall LPL expression is indeed decreased, but lipid hydrolytic and endocytic rates at sebaceous glands may approach those of fed rates, despite lower lipid levels, due to increased specific activity and to the contrariwise, dramatic rate reductions in adipose tissue. Therefore, a non-fasting-induced decrease of plasma lipids as caused by a systemic increase in LPL could cause a clinically significant decrease in sebogenesis. Thus, LPL-replacement therapy could be an effective means to lower SERs; and, hence, could be used to treat seborrhea.

This theory of treatment is similar to that for obesity which is caused by the overactivity of the various correlated pathways of adipose tissue lipogenesis, wherein LPL may also be paradoxically indicated. For, LDL and fatty acid uptake - via lipoprotein receptors (LDL) and FA transporters (FATP4) of the sebaceous glands - figure prominent in sebogenesis. And these are byproducts of LPL-mediated hydrolysis, as are PARR ligands. Nevertheless, in the case of both obesity and seborrhea, regardless of pre-existing LPL levels, a relative decrease of the exogenous lipid pool could result in a net decrease in lipid accumulation by adipocytes and sebocytes. Therefore, a systemic increase in LPL could effectively decrease adipocytic, as well as sebocyte, differentiation.

LPL has the same relationship with a variety of specific pathologies, where in vitro selective expression is contraindicative, but, in practice, systemic underexpression is proindicative. Among these are renal disease, pancreatitis, hepatosteatosis, atherosclerosis, and seborrhea.

Fibrates, thiazolidinediones, and statins - even though they may reduce plasma lipids and increase LPL expression, they do not reduce sebum production; rather, they cause its net increase,
as may be seen in their potential seborrheic side-effects. This is due to their independent and synergistic upregulation of peroxisome proliferator-activated receptors (PPARs), which have been shown to regulate sebum production. It has been found that fibrates and thiazolidinediones increased sebum production by 77% and 37%, respectively; and this increase was directly linked to PARR stimulation. It is probably as PARR ligands that these drugs also upregulate the synthesis of LPL, since PARR is a LPL-transcription factor. Therefore, in refractory cases, the present invention may be used in conjunction with these drugs, since there could be an additive and synergistic effect on plasma lipid clearance and LPL-replacement would counter promiscuous PARR stimulation in sebaceous glands.

The currently available therapies that express LPL comprise fibrates, statins, thiazolidinediones, and other more selective LPL-activator drugs, like NO-1886; and bio-engineered gene-replacement therapies, as in AMT-011. Simple replacement therapy with bovine milk LPL has been shown to be infeasible, due to the short half-life of the LPL post-injection, with respect to the cost of such a treatment regimen. No LPL-replacement therapy has yet been invented that is safe, inexpensive, convenient, and effective. Milk LPL would likely work, particularly bovine milk, but a feasible method of use has not yet been discovered. The present invention comprises the nutraceutical use of LPL by oral administration, which has never before been proposed.

Therefore, one aspect of present invention includes the pharmaceutical use by oral administration in mammals of any kind of LPL, so long as it is effective, although animal LPL - and bovine milk LPL, in particular - is preferred in humans and wherever else it is effective. During lactation, mammary tissue overexpresses LPL to upregulate the local metabolism of plasma lipids for the production of milk. The highly productive LPL isozyme expressed in the mammary tissue is the same as occurs in milk. However, milk LPL secretion is not collateral to the secretion of milkfat, for the LPL content of milk is independently correlated with the fat content, which is dependant on the stage of lactation and the species. (For instance, human and bovine milk is on average 4.5% fat; rat, 15%; rhinoceros, 0%; and seal, 53.2%). Nor is the secretion of LPL due to leakage, since agitation during milking does not increase its expression and the occurrence of serum albumin and somatic cells in milk, which are indicators of leakage, may be negatively correlated with the LPL. Rather, LPL has been shown to be as efficiently
secreted as other milk proteins. This suggests that the LPL content of milk is not accidental or random, but meets the specific need of sucklings for the metabolism of the milk fat at every stage of development throughout lactation.

The LPL needs to be able to survive in the GI tract and be absorbed in tact into circulation. But many bioactive constituents of milk are designed to survive digestion because they are needed to compensate for the immature functions in neonates. The calf abomasum and human stomach have a normal pH of about 1.5, which is too acidic for LPL to remain active. However, milk is a buffer solution of pH 6.6 and, so, initially resists an increase in acidity. As for the prolonged survival of essential bioactive material that might remain in the stomach, gastric acid is also inhibited by casein. Specifically, as casein enters the stomach, aspartic proteases competitively act on calcium-soluble κ-casein (169 amino acids) at the bond that joins its water-soluble and insoluble parts. This produces a hydrophobic remnant, para-κ-casein (1-105), that remains with the micelle, and a hydrophilic fragment, glycomacropeptide (GMP) (106-169), that dissolves in the serum. However, the remnant is no longer calcium-soluble. Therefore, since the concentration of calcium required for micelle formation is greater than that required for the precipitation of αs-casein isotypes at physiologic conditions, most casein precipitates from the milk serum by calcium. It is the first proteolytic product, GMP, that has been shown to inhibit gastric acid and motility. The bioactive components of milk are water-soluble and mostly proceed ahead of the clotted casein into the alkaline intestine; but casein micelles are highly solvated and contain 3.7 times as much water as protein. Within about 15 minutes of feeding, a calf’s abomasal pH is 6 and stays above 5½ for about an hour. The pH of the intestine (7-8) is well-tolerated by LPL.

Therefore, with respect to the whole milk, skim milk, and reconstituted forms of the present invention, a minimum dosage is believed to be only required to maintain the activity of LPL as it passes through the stomach. Furthermore, in more purified forms of the present invention, a lack of κ-casein may be made up for by the addition of other acid inhibitors or by the use of an enteric carrier; however, this is not required.

LPL is glycosylated and tightly folded and, thus, intrinsically resistant to proteolysis. Other milk proteins, by these same features, have been shown to resist proteolysis by digestive proteases native to both the stomach and intestine. However, direct evidence on whether LPL,
itself, may survive under in vivo conditions does not exist. Gastric or aspartic proteases, though, are commonly used in milk LPL purification processes with no loss of function or activity. On the other hand, it has been shown that isolated bovine LPL, when incubated in vitro with serine proteases native to the intestine - namely, trypsin, chymotrypsin, and plasmin - it is truncated (≤390-391) such that it loses the ability to interact with insoluble long-chain triglycerides, like those found in lipoproteins. However, milk contains plasmin - as well as plasminogen and plasminogen activators - which most have a strong affinity for casein, with which LPL also is closely associated; but, whereas plasmin does act on the casein - a process important for ripening of some cheeses - it apparently does not act on the LPL - even when these are precipitated with casein from the serum. For milk does contain a variety of serine protease inhibitors, but these reside in the serum fraction to putatively protect whey proteins when in the intestine. As such, LPL is believed to be protected from plasmin by competitive proteolysis of the casein. Plasmin seems to have the highest specificity for β-casein (penultimate ref), with κ-casein having been reported to be resistant. Therefore, also, in the present invention, the nutraceutical and pharmaceutical compositions may mostly contain β-casein to ensure no loss of function due to serine proteases in the intestine.

Clinical evidence in favor of the assimilation of active LPL into circulation and equally of the efficacy of bovine LPL as a nutraceutical in humans formost the aforementioned conditions is that cow’s milk used to be orally administered by doctors in this country for the treatment of a variety of pathologies, including cardiovascular disease, Bright’s disease (renal diseases), dropsy (edema), obesity, hypertension, and diabetes; tuberculosis, gastritis, cystitis, various infections, and rheumatic fever; and psoriasis and generally anti-cosmetic indications of the skin. These are many of the same pathologies associated in modern times and in the present invention with LPL deficiency. In fact, the wide variety of pathologies that used to be treated by milk may be separated under three main umbrella conditions: metabolic syndrome, seborrhea, and immunodeficiency. Exactly why milk was therapeutically effective was not known; but it appears that it may have had to do with raw milk’s active LPL content with regard to the first two conditions; and that immunodeficiency is ameliorated by its active antimicrobial, immunomodulatory, and anti-inflammatory ingredients. LPL also has anti-inflammatory effects.

A specific embodiment of Certified Milk treatment, as employed at a sanitarium run by
Dr. Crewe of the Mayo Clinic, which he published in 1929, comprised the use of Certified Guernsey milk (700 calories per quart), orally administered every half hour in small dosages, totaling 5-10 quarts per day. Most patients were started on 3-4 quarts per day, which was usually increased 1 pint per day. This was accompanied by bed confinement, diaphoresis, and a daily enema. Treatment durations varied by case. Dr. Alan Howard, inventor of the Cambridge Diet for weight and blood cholesterol control, claimed that 2 quarts of whole milk a day is sufficient to significantly lower total blood cholesterol. Dr. George Mann claimed that 4 quarts of whole milk per day lowered blood cholesterol levels by 25%. After the introduction of the pasteurization of milk, its clinical use began to fall into disfavor with doctors and the public, in general, as it was realized that milk's therapeutic property was dependent on it not being heat treated. According to the USFDA, the minimum temperature/time requirements for the pasteurization of milk are: 63°C (145°F)/ 30 minutes, 72°C (161°F)/ 15 seconds, etc. The temperature of conformation conversion of bovine LPL in milk is 41.5°C.; and heating milk at 70 and 75°C., for 15 seconds, results in a 98 and 100% reduction in LPL activity, respectively.

Although various non-heat dependant methods have been proposed to depathogenize milk, as in microfiltration, centrifugation, and peroxide use, the USFDA has not yet approved any of them as safe and certain alternatives to heat treatment. Therefore, in the U.S., pasteurization is required of almost milk except where the sale of raw milk is permitted. However, even if one of the above methods were approved for use, the prevalence of the infrastructure for pasteurization and its established cost effectiveness would still call for a way to thermostabilize LPL in milk. Moreover, even if a cheaper way to depathogenize milk than pasteurization did exist or the federal ban on the sale of raw milk were lifted, thermostable milk would still be desirable for its use in cooking. Furthermore, currently the cheapest way to mass produce powdered milk or milk proteins - as for encapsulation or for use as a food additive - is by spray-drying, which process would deactivate non-stabilized milk LPL.

One aspect of the present invention involves a process to afford milk to the masses whereby guidelines for precautionary depathogenization may be followed without the loss total loss of milk's natural nutracoology, by the thermostabilization of the LPL content.

The science behind the art is sufficiently advanced that many ways may be devised to
thermally stabilize an enzyme; and various methods have been invented that specifically apply to LPL. However, these methods concern themselves with highly purified versions of lab-

popular microbial LPL. Takeda et al. was issued a patent in 1993 on a method that thermostabilized streptomyces-derived LPL capable of fully resisting denaturation at 60° C. for 15 minutes, while treated in a buffer. This method was based on the discovery of a naturally occurring microbe that produced the thermostable LPL when it was extracted by certain known means. Minoshima et al. was issued a patent in 2002 for a thermostable microbial LPL composition, comprising LPL and certain known thermostabilizing agents, which was prepared by ways known in the art as effective. The microorganism producing the LPL was not limited. Upon subjection to tests at 80° C. for 0, 10, 20, and 30 minutes, the activity of said composition lost just 15% of its starting activity in the final test. Furthermore, the composition purportedly stood up to spray-drying and, in a reconstituted form, had comparable results for the same tests: with desirable upper limits of the drying process being 20,000 rpm for the atomizer, 200° C. for the inlet temperature, and 100° C. for the outlet temperature. The preferred embodiment of the thermostabilization process comprises: purification of the LPL from the culture by centrifugation, microfiltration, etc. and dilution; then, mixing of the aqueous LPL solution with a thermostabilizing agent, which comprises a phospholipid and a lipophilic vitamin.

The present invention is directed to a process to thermostabilize LPL in bovine milk and has taken into account both the various competitive and inhibitory factors of the milk to potential thermostabilizers and the need to also preserve and enhance antdigestive factors for the substantial assimilation of the LPL. The LPL in bovine whole milk is associated with the casein micelle fraction rather than with phospholipids, as less than 3% of the total LPL activity can be found with the MFGM and fluff, which are where most of the phospholipids in milk are contained. Although, the LPL is present in higher concentrations in these phospholipid-rich fractions, which suggests potential. Fluff is a moiety that dispersed throughout the main fractions and obtainable after settling takes place. Furthermore, milk already has numerous highly lipophilic food microconstituents (HLFMs) that might act as thermostabilizers - including vitamins A, E, D, K, carotenoids, and phytosterols - but these are embedded in the milkfat fraction and therefore do not interact with LPL. As for antdigestive factors in bovine milk for LPL, these essentially consist in the acid inhibition and competitive proteolysis of casein. Also
important are the buffering agents in milk which resist increasing acidity on first contact with stomach acid. These are calcium salts, which include casein, phosphorus, and citrate; casein is the most important buffering agent in milk. Diffusible calcium (Ca\(^{2+}\)) may also act as a stabilizer for milk LPL. Ca\(^{2+}\) has also been shown to initiate the dimerization of LPL monomers and reverse deactivation. The addition of Ca\(^{2+}\) in the present invention is considered desirable, but not required. That casein has been shown to be the essential antiedigestive factor in milk for LPL is convenient in that due to its characteristic native conformation, casein is thermostable.

One non-limiting aspect of the present invention is directed to the process for the thermostabilization of milk, wherein the skim milk containing most of the LPL in bovine milk is warm separated, up to or near about 40° C., and, then, the skim milk is cooled, down at or close to about 0° C., whereupon the LPL is commixed with stabilizers naturally present in the skim milk, along with optionally added LPL stabilizers. Buffering agents may also be added to reduce the minimum dosage required for the survival of LPL through the stomach.

Conventional whole milk, currently, undergoes a process that generally comprises a duel clarification and separation step, with a standardized recombination of the cream and skim milk, homogenization, and pasteurization. This is followed by packaging. Increasingly, milk plants are equipping to partially homogenize the cream before standardization, as a way to conserve energy and save money. However, since homogenization breaks the membrane that coats milk fat globules in order to protect them from the action of LPL, homogenized milk fat cannot be added to skim milk containing active LPL, as this would lead to rapid rancidification. Therefore, in another non-limiting aspect of the present invention the present invention, the whole milk is not homogenized.

The present invention, in still another non-limiting embodiment, comprises duel clarification and separation, partial homogenization of the skim milk, where known stabilizers of LPL are added, followed by standardization, pasteurization, and packaging. Partial pasteurization of both parts may also precede standardization; however, this is not required.

In human milk, LPL is associated with the milkfat globules; but in bovine milk, it is associated with the casein micelles. According to Hohe et al., in raw bulk tank milk, about 74% of the LPL is in the casein micelle fraction; 25%, in the serum; and 0.6%, in the MFGM. Chilliard et al., using milk buckets to obtain their samples, found the relative distribution to be
78, 17, and 6%, in the casein, whey, and cream, respectively - allowing for a higher concentration in the casein if a less agitative milking method were used. Therefore, in the present invention, it is desirable, but not required, to use the most gentle milking and processing methods possible, at least until a final form be obtained, in order to maximize its specific activity.

Milk readily separates into three (3) main fraction, namely: cream, serum (whey proteins), and casein. The cream consists mostly of milk fat globules encased in a phospholipid-rich membrane (MFGM). Cream may be separated from the heavier milk proteins by centrifugation, as with a mechanical cream separator, or simply by gravity. Casein may be separated from whey by rennet precipitation and subsequent drying, as by straining and/or heating (called cooking). Enzymatic precipitation is desirable, but not required, over pure acid precipitation in the present invention because the isoelectric point of casein (4.6) is too low for LPL to keep active. Bovine milk contains 24-29 g/L of casein. Lactose does not associate with casein. Thus, another non-limiting aspect of the present invention includes a nutracologic fat-free skim milk and a fat and lactose-free casein protein isolate that may be simply and inexpensively prepared utilizing known methods.

A high degree of purity, however, is not absolutely necessary for the efficacy of the present invention. Indeed, whole milk, itself, in many cases, will provide a net nutracologic benefit. Therefore, the present invention can include the nutracologic use of a whole milk. Skim milk would be highly amenable to nutracologic use; although, it may be contraindicated for serious cases of diabetes, obesity, and lactose intolerance. Lactose represents milk’s sugar and carbohydrate content. Therefore, merely removing lactose from skim milk would make it and its derivatives sugar and carb-free. This is usually done by ultrafiltration for the retentate. As for milk specifically for the lactose intolerant, lactase - a variety with a non-acidic optimum pH - may be mixed into the milk product as is usually done, which breaks down the lactose into its subunits, glucose and galactose. But the most convenient and potentially purest form of milk LPL would be in a pill form, which the present invention also contemplates. However, in producing a medicinal composition of purified milk LPL, its separation from natural antiproteases, stabilizers, and buffers in milk should be taken into account in order to prevent its denaturation during digestion.

Casein is a family of phosphoproteins comprised of four (4) primary heterogeneous
isotypes: \(\alpha_s 1\), \(\alpha_s 2\), \(\beta\), and \(\kappa\). Under physiologic conditions, the isotypes in milk coalesce into micelles in a way that appears to be largely dependant on isotypical proportions and on the genotypes that occur thereof; thus caseins display a memory when dissociation is induced and then they are permitted to re-associate. In other words, for a given set of conditions, casein micelles comprise definite ratios of caseins. Therefore, depending on the peculiar casein content in some sample of milk, certain caseins will have had been unable to associate with micelles and will occur in the serum phase. The odd casein out, most of the time, would appear to be the more water-soluble caseins, \(\kappa\) and \(\beta\)-casein, inasmuch as these are the ones most readily dissociated from the micelle. For instance, the addition of \(Ca^+\) to milk is known to associate almost all the casein with micelles, but the addition and removal of \(Ca^{2+}\) is also known to especially associate and dissociate the water-soluble caseins. This illustrates that at physiologic conditions, not all the casein is with micelles; and that most of the free casein is water-soluble. Now, bovine milk contains a relatively low proportion of \(\kappa\) and \(\beta\)-casein (38%), compared to caprine (55%) and human (70%). This, then, could account for the relatively high proportion of LPL associated with the casein micelle fraction of bovine milk (>80%); the even distribution of LPL in the serum and cream in caprine (46%/46%); and the high proportion of LPL associated with the cream in human. For, LPL also has a known affinity for phospholipids, which the MFGM is replete with. Therefore, as milk incubates, whether in or out of the udder, free \(\kappa\) and \(\beta\)-casein could gradually lose LPL to the MFGM. Thus, if LPL were initially associated with \(\kappa\) and/or \(\beta\)-casein, then, to the extent that some milk contained free \(\kappa\) and \(\beta\)-casein, which would be directly related to its proportion of them, it would have also LPL that were not associated with casein micelles. Furthermore, \(\kappa\) and \(\beta\)-casein is also known to salt out or solubilize in refrigeration and dissociate from micelles and contribute to serum casein. If LPL were associated with \(\kappa\) and/or \(\beta\)-casein, this would also explain the phenomenon of spontaneous lipolysis, which happens to predisposed fresh bovine milk when it is cooled and LPL somehow migrates to the MFGM.

Therefore, the present invention assumes that LPL is initially associated with \(\beta\)-casein and makes use of the salting in and out properties of the soluble caseins in order to make various protein isolates that comprise most of the LPL in bovine milk. Such isolates are advantageous because they include the natural antiprotease for LPL, \(\beta\)-casein, and have high specific activities, making them highly amenable for use as additives or to put in pill form. These isolates in pill
form would require acid inhibitors for the survival of the LPL through the stomach or, as is preferred in the present invention, an enteric carrier.

Still another non-limiting aspect of the present invention is directed to a milk soluble protein (MSP) isolate prepared from the skim milk of the present invention, where the micellar calcium phosphate (MCP) of said milk is removed, as by the chelation of the calcium and subsequent removal of the chelate complexes. The chelating agent may be any non-toxic substance such as a sodium carbonate or sodium citrate. The chelator is added in a concentration where no more κ and β-casein may be dissociated from micelles at a given temperature, down to or near about 0° C., to which temperature the skim milk is also brought; and the skim milk is agitated, as by homogenization; then, the casein micelles are precipitated by rennet; and the precipitate is removed and the entrapped water is extracted back into the aqueous phase. The water-soluble proteins may then be spray-dried and encapsulated; however, this is not required.

Still yet another non-limiting aspect of the present invention is directed to a casein isolate prepared from the starter skim milk of the present invention, where calcium is mixed into said milk at or near the concentration where no more κ and β-casein may be associated with the micelles at or near about 40° C., to which temperature the skim milk is also brought; then rennet is mixed in to precipitate the casein micelles at or near about 40° C.; the precipitate may then be spray-dried and encapsulated; however, this is not required.

Another non-limiting aspect of the present invention is directed to a soluble casein isolate prepared from the casein isolate of the present invention described above, where said precipitate is dispersed in demineralized water at or near a concentration wherein the highest amount of κ and β-casein may be finally solubilized; the MCP of the dispersion is removed as by the chelation as described above; and the coagulated casein is broken up and the dispersion is agitated, as by high pressure homogenization (HPH); then, the solution is separated from the insoluble casein, as by centrifugation or filtration; the solution may then be spray-dried and encapsulated; however, this is not required.

Still another non-limiting aspect of the present invention involves a milk soluble protein (MSP) isolate prepared from the skim milk of the present invention, where the micellar calcium phosphate (MCP) of the milk is solubilized, as by the chelation of the calcium; and the chelate complexes may be subsequently removed, as by skimming, straining, or filtration. The chelating
agent may be any non-toxic substance such as a sodium carbonate and/or sodium citrate. The chelator is added in a concentration where no more \( \kappa \) and \( \beta \)-casein may be dissociated from micelles at a given temperature, down to about 0\( ^\circ \) C, for a given incubation period (e.g. 50 milimoles per liter of trisodium citrate at 30\( ^\circ \) C for 1 minute), to which target temperature the skim milk is also brought and closely maintained at the lower the temperature the more soluble the caseins become and the less calcium needed to induce complete solubilization. Then, the skim milk is agitated, as by homogenization, so as to speed up the solubilization process, but at a temperature not rising substantially above the target maximum temperature of the prior step, so as not to allow any caseins to substantially re-insolubilize or salt back out. Then, the casein micelles are precipitated by rennet - also at the target temperature - and the precipitate is removed; and the entrapped water is extracted back into the aqueous phase. The water-soluble proteins of the aqueous phase may then be spray-dried and encapsulated.

Still yet another non-limiting aspect of the present invention involves a casein isolate prepared from the starter skim milk of the present invention by any known process for doing so, where the improvement comprises the addition of calcium to said milk in a concentration where no more \( \kappa \) and \( \beta \)-casein may be associated with the micelles at a given temperature (e.g. 150 milimoles per liter of calcium chloride at 37\( ^\circ \) C.), to which target minimum temperature the skim milk is also brought; then rennet is mixed in to precipitate the casein micelles, at a temperature not falling substantially below the target minimum temperature, so as not to allow a substantial solubilization of caseins; the precipitate is removed, which may then be spray-dried and encapsulated. And the present invention comprises a soluble casein isolate prepared from the casein isolate of the present invention described above, where said precipitate is dispersed in demineralized water at a concentration of 1 to 25% or the optimum concentration where the highest amount of \( \kappa \) and \( \beta \)-casein may be finally solubilized; the MCP of the dispersion is removed as by chelation, as described above; and the coagulated casein is broken up and the dispersion is agitated, as by high pressure homogenization (HPH) - so as to promote and speed up the dissociation and solubilization of the more readily solubilized caseins - and where the target maximum temperature is not substantially exceeded. Then, the aqueous phase is separated from the insoluble casein, as by centrifugation or filtration, also at a temperature not substantially exceeding the target maximum. The solution may then be spray-dried and encapsulated. The
improvement comprises the addition of calcium to said milk in a concentration where no more κ and β-casein may be associated with the micelles at a given temperature.

Another non-limiting aspect of the present invention involves a process to increase the thermostability of LPL in milk by utilizing the buffering and stabilizing agents already present therein - including, for example, phosphorus, albumin, lactose, calcium, casein, and the lipophilic vitamins. One non-limiting embodiment of the method in accordance with the present invention comprises:

- separation of raw whole milk for the skim milk fraction (e.g., with a separator, microfilter, etc.);
- homogenization of the skim milk fraction which contain the LPL and native stabilizers along with optionally added lipophilic vitamins, as a large portion of them will have been lost with the cream moiety for which they have an affinity, or other stabilizers, until the LPL is sufficiently thermostable;
- and, then, optional pasteurization, as this may not be desired or required;
- from here, the skim milk may be used as is, flavored, dried, powdered, evaporated, recombined with the cream, added to other foods, or processed in some other way and combinations thereof.

One non-limiting reason that it is desirable to mix up the LPL in absence of the cream is because the milk fat, a natural substrate of LPL, when agitated, largely loses the native membrane it has that protects it from the lipolytic action of the LPL. So, no prior separation could tend to lessen the thermostabilizing effect of mixing and increase the lipolytic flavor of the product and, not to mention, destabilize the milk fat.

One may test the effectiveness of the inventive process by subjecting a product of the process to heat extremes and then introducing to it triglyceride (TG) oil to see if the LPL acts on it by testing for free fatty acidity.

Active LPL, when in the presence of triglyceride and water, will catalyze lipolysis. That is, it will catalyze the split of triglyceride into its constituent glycerol and three fatty acids by the action of water. Also, LPL is the preponderant catalyst for lipolysis in milk. Therefore, a free fatty acidity test of milk is an excellent indicator of the presence of active LPL.

A TG oil emulsion can be prepared by cold blending extra virgin olive oil, 4% polyvinyl
alcohol, and ice cubes of deionized water (1:1:4, respectively) until emulsified.

Fresh, warm raw milk can be separated using a standard benchtop separator.

The skim milk fraction can be blended at about 5000-25000 rpm's (typically about 15,000 rpm's) at least about 0.5 minutes and typically at least about 5 minutes.

The blended skim milk can then be poured into 4 glass beakers, in equal amounts.

One beaker can be placed in a water bath of about 72°C for about 15 seconds.

The other beaker can be spray dried with the contents of another beaker and then reconstituted with deionized water back in a glass beaker.

The 2 beakers and a 3rd beaker can be placed in a water bath of about 40°C and admix to each the same amount of the TG oil emulsion; allow about 15 minutes for the reaction, keeping the temperature constant. Do nothing to the contents of the 4th beaker.

An equal part of alizarin alcohol can be added to each the 4 beakers and compare against a colorimeter.

The results should show that the thermostability of the lipase in the mixed milk samples is high enough to withstand pasteurization and spray drying.

The present invention can also involve, in particular, a process for the production of whole milk, with thermally stable LPL, that is useful for the intervention and prevention of metabolic syndrome and seborrhea and their pathogonies, comprising:

- the separation of raw whole milk into cream and skim milk;
- optional homogenization of the cream,
- homogenization of the skim milk with optionally added lipophilic vitamins or other stabilizers, until the LPL is sufficiently thermostable;
- standardized recombination of the cream and skim milk;
- and optional pasteurization;
- from here, the milk may be readied for distribution in any number of ways.

The present invention also can involve a process for the production of a dietary supplement containing LPL, which is useful in the intervention and prevention of metabolic syndrome and seborrhea and their pathogonies, comprising:

- the separation of raw whole milk for the skim milk fraction as with a milk separator or microfilter;
homogenization of the raw skim milk along with optionally added lipophilic vitamins or other stabilizers, until the LPL is sufficiently thermostable;
- optional pasteurization;
- and ultrafiltration with diafiltration for the milk protein retentate;
- from here, the milk protein may be used as is or further concentrated or powdered in any number of ways and used as a food additive or put into pill form.

It will thus be seen that the objects set forth above, among those made apparent from the preceding description, are efficiently attained, and since certain changes may be made in the constructions set forth without departing from the spirit and scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense. The invention has been described with reference to preferred and alternate embodiments. Modifications and alterations will become apparent to those skilled in the art upon reading and understanding the detailed discussion of the invention provided herein. This invention is intended to include all such modifications and alterations insofar as they come within the scope of the present invention. It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described and all statements of the scope of the invention, which, as a matter of language, might be said to fall therebetween.
I claim:

1. The use of edible lipoprotein lipase for the intervention and prevention of metabolic syndrome and seborrhea and their pathogomies.

2. A nutraceutical or pharmaceutical composition for the treatment of metabolic syndrome and seborrhea and their pathogomies, comprising milk or one of its fractions or derivatives, where said composition contains active and/or stabilized lipoprotein lipase.

3. A nutraceutical or pharmaceutical composition for the treatment of metabolic syndrome and seborrhea and their pathogomies, comprising a skim milk that contains active and/or stabilized lipoprotein lipase.

4. The composition as defined in claim 2 or 3, wherein said composition is made into a powder, as by evaporation, spray-drying, or freeze-drying.

5. The composition as defined in any one of claims 2-4, wherein said composition is recombined in some proportion with non-homogenized milk fat.

6. The composition as defined in any one of claims 2-5, wherein said composition is made into a powder, as by evaporation, spray-drying, or freeze-drying.

7. The composition as defined in any one of claims 2-6, wherein said composition is further processed to make a milk soluble protein isolate.

8. The composition as defined in any one of claims 2-7, wherein said composition is made into a pill with a suitable carrier.

9. The composition as defined in any one of claims 2-8, wherein said composition is further processed to make a casein isolate.
10. A process for the preparation of milk or milk fraction that contains active and/or stabilized lipoprotein lipase comprising the steps of:
   a. warm separate cream;
   b. cooling of skim milk; and,
   c. homogenizing the cooled skim milk.

11. The process as defined in claim 10, wherein said step of warm separation of cream includes heating cream up to about 45° C.

12. The process as defined in claim 10 or 11, wherein said step of cool of skim milk includes cooling said skim milk to about 0-25° C.

13. The process as defined in any one of claims 10-12, including the step of adding buffering and/or stabilizing agents to said homogenize cooled skim milk for at least 1 minute and up to about 30 minutes until LPL in said skim milk is thermostable.

14. The process as defined in any one of claims 10-13, wherein said milk or milk fraction includes, skim milk, milk containing non-homogenized milk fat, milk soluble protein isolate, casein isolate, soluble casein isolate, and combinations thereof.

15. The process as defined in any one of claims 10-14, including the step of recovery of soluble casein.

16. A process for the preparation of a milk soluble protein isolate comprising the following steps:
   a. solubilizing micellar calcium phosphate in milk or milk fraction that contains active and/or stabilized lipoprotein lipase by the mixing in a chelating agent;
   b. agitating said milk or milk fraction that contains active and/or stabilized lipoprotein lipase;
   c. precipitating casein micelles from said milk or milk fraction that contains active
and/or stabilized lipoprotein lipase;

d. extracting water from said precipitated casein micelles to form dried casein micelles; and,

e. add said dried casein micelles to said milk or milk fraction that contains active and/or stabilized lipoprotein lipase.

17. The process as defined in claim 16, wherein said chelating agent includes trisodium citrate, sodium carbonate, sodium citrate, and combinations thereof.

18. The process as defined in claim 16 or 17, wherein said step of solubilizing micellar calcium phosphate includes cooling said milk or milk fraction that contains active and/or stabilized lipoprotein lipase to about 0-25° C.

19. The process as defined in any one of claims 16-18, wherein said step of solubilizing micellar calcium phosphate includes adding said chelating agent at a concentration of about 50 milimoles per liter about 25-40° C for at least about 10 seconds wherein little or no more κ and β-casein can be solubilized.

20. The process as defined in any one of claims 16-19, including the step of solubilizing chelate complexes from said milk or milk fraction that contains active and/or stabilized lipoprotein lipase.

21. The process as defined in any one of claims 16-20, wherein said step of agitating is at least partially by homogenization to about 0-25° C.

22. The process as defined in any one of claims 16-21, wherein said step of precipitating said casein micelles is by rennet at or near about 0-25° C.

23. A process for the preparation of a casein isolate comprising the steps of:

a. mixing calcium to milk or milk fraction that contains active and/or stabilized
lipoprotein lipase; and,
b. precipitating casein micelles from said milk or milk fraction that contains active
and/or stabilized lipoprotein lipase.

24. The process as defined in claim 23, wherein said step of mixing includes adding
said calcium at or near a concentration wherein little or no more $\kappa$ and $\beta$-casein can be
insolubilized at a given temperature up to about 45°C.

25. The process as defined in claim 23 or 24, wherein said step of precipitating said
casein micelles is by rennet.

26. A process for the preparation of a soluble casein isolate comprising the steps of:
a. dispersing casein isolate in demineralized water at or near a concentration wherein
a highest amount of $\kappa$ and $\beta$-casein is solubilized;
b. solubilizing micellar calcium phosphate from said dispersion by adding a
chelating agent;
c. breaking up said dispersion via agitation; and,
d. separating an aqueous phase from an insoluble phase in said dispersion.

27. The process as defined in claim 26, wherein said step of separating includes
centrifugation, filtration, or combinations thereof.

28. The process as defined in claim 26 or 27, wherein said chelating agent includes
trisodium citrate, sodium carbonate, sodium citrate, and combinations thereof.

29. The process as defined in any one of claims 26-28, wherein said step of
solubilizing micellar calcium phosphate includes cooling said dispersion to about 0-25°C.

30. The process as defined in any one of claims 26-29, wherein said step of
solubilizing micellar calcium phosphate includes adding said chelating agent at a concentration
wherein little or no more κ and β-casein can be solubilized.

31. The process as defined in any one of claims 26-30, including the step of optionally removing chelate complexes from said dispersion.

32. The process as defined in any one of claims 26-31, wherein said agitation is at least partially by homogenization at about 100 to 10,000 rpm.

33. A food, BIOGALACT™ useful for the intervention and prevention of metabolic syndrome and seborrhea and their pathomogies, comprising skim milk with active, thermostable LPL.

34. A food useful for the prevention of metabolic syndrome and seborrhea and their pathomogies, comprising standard whole milk with active, thermostable LPL and/or BIOGALACT™.

35. A dietary supplement useful for the intervention and prevention of metabolic syndrome and seborrhea and their pathomogies, comprising a protein isolate form of active, thermostable LPL and/or BIOGALACT™.

36. The supplement as defined in claim 35, wherein said supplement is further powderize.

37. The supplement of claim 35 or 36, wherein said supplement is encapsulated in a dosage more or less equal to the amount in the recommended serving of milk or one that is efficacious.

38. A method for the intervention or prevention of the metabolic syndrome and seborrhea and their pathomogies comprising the oral administration of the food as defined in any of claims 33-37 in a daily amount more or less equal to the recommended allowance of milk,
wherein the amount is efficacious.

39. A method for the intervention or prevention of xanthomatosis comprising the oral administration of the food as defined in any of claims 33-37 in a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

40. A method for the intervention or prevention of atherosclerosis comprising the oral administration of the food as defined in any of claims 33-37 in a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

41. A method for the intervention or prevention of seborrhea and its pathomologies effects comprising the oral administration of the food as defined in any of claims 33-37 in a daily amount more or less equal to the recommended allowance of milk, wherein the amount is efficacious.

42. A method for the intervention or prevention of acne comprising the oral administration of the food as defined in any of claims 33-37 a daily amount more or less equal to the recommended allowance of milk, where the amount is efficacious.

43. A method for the intervention or prevention of eczema or psoriasis comprising the oral administration of the food of as defined in any of claims 33-37 in a daily amount more or less equal to the recommended allowance of milk, where the amount is efficacious.

44. A process for the production of the food as defined in any of claims 33-37, comprising:
   separating raw whole milk to form the skim milk fraction, said step of separating including the use of separator, microfilter or combinations thereof;
   homogenizing of the skim milk fraction along with the optional addition of lipophilic vitamins or other stabilizers, until the LPL is at least partially thermostable;
   optionally pasteurizing the homogenized skim milk fraction.
45. A process for the preparation of a casein isolate comprising the steps of:
   a. mixing calcium to milk or milk fraction that contains active and/or stabilized lipoprotein lipase; and,
   b. precipitating casein micelles from said milk or milk fraction that contains active and/or stabilized lipoprotein lipase.

46. The process as defined in claim 45, wherein said step of mixing includes adding said calcium a concentration of about 50-200 milimoles per liter wherein little or no more κ and β-casein can be insolubilized at a given temperature of about 35-45° C, said calcium including calcium chloride.

47. The process as defined in claim 45 or 46, wherein said step of precipitating said casein micelles is by rennet.
### INTERNATIONAL SEARCH REPORT

**A. CLASSIFICATION OF SUBJECT MATTER**
- IPC(8) - A61K 31/336, A23J 1/00 (2008.04)
- USPC - 514/475, 426/580

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**
- Minimum documentation searched (classification system followed by classification symbols)
  - IPC(8) - A61K 31/336, A23J 1/00 (2008.04)
  - USPC - 514/475, 426/580

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
- PubWEST (USPT, PGPB, EPAB, JPAB); Google Patents; Google Scholar
- Search Terms: lipoprotein, lipase, dry, micelle, casein, metabolic syndrome, aggregate, calcium chloride

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>US 8183803 B1 (MORCOL et al.) 06 Feb 2001 (06.02.2001), col 3, In 1-17;</td>
<td>16-18, 23-28, 45-47</td>
</tr>
<tr>
<td>Y</td>
<td>US 5,942,500 A (PERRY) 24 Aug. 1999 (24.08.1999), col 1, In 6-12; abstract,</td>
<td>33-37</td>
</tr>
</tbody>
</table>

- **X** document defining the general state of the art which is not considered to be of particular relevance
- **E** earlier application or patent but published on or after the international filing date
- **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- **O** document referring to an oral disclosure, use, exhibition or other means
- **P** document published prior to the international filing date but later than the priority date claimed
- **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- **X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- **Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- **&** document member of the same patent family

**Date of the actual completion of the international search**
- 23 Sep. 2008 (23.09.2008)

**Date of mailing of the international search report**
- 29 SEP 2008

**Name and mailing address of the ISA/US**
- Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
- P.O. Box 1450, Alexandria, Virginia 22313-1450
- Facsimile No. 571-273-3201

**Authorized officer**: Lee W. Young
- PCT Helpdesk: 571-272-4300
- PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)
**INTERNATIONAL SEARCH REPORT**

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant’s protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant’s protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2007)