This invention relates to metal-embedded, insoluble hydrolyzed proteins and fragments thereof derived from pooled adipocytes and formulated for oral delivery. A composition, a method of making, as well as a method for treating and/or preventing atherosclerosis, obesity, and obesity-related disorders such as diabetes are provided.
COMPOSITION FOR ATHEROSCLEROSIS, OBESITY AND OBESITY-RELATED DISORDERS

RELATED APPLICATIONS

This application claims priority to two U.S. provisional applications: 61/291,405, filed on December 31, 2009 and 61/292,666, filed on January 6, 2010.

FIELD OF THE INVENTION

The present invention discloses a composition useful in the treatment or prevention of atherosclerosis, obesity, and obesity-related disorders such as diabetes. Methods of treating said conditions are also disclosed. More particularly, the composition of the present invention derives from adipose tissue or adipocytes and comprises pooled antigens such as proteins and fragments thereof. The invention is pertinent to the field of vaccines and immune therapies.

BACKGROUND OF THE INVENTION

Coronary heart disease (CHD) is the main cause of death in developed countries. Atherosclerosis and obesity are two principal pathological conditions that predispose to cardiovascular disease. The term atherosclerosis, commonly referred to as a "hardening of the arteries," is associated with the formation of lipid-laden atheromas or plaques within the arteries. Excessive body fat accumulation characterizes overweight and obesity - two pathological conditions - which according to the WHO, affects two billion adults worldwide. Epidemiological studies have shown that high levels of atherogenic low density lipoproteins (LDL - also known as "bad cholesterol") and triglycerides (TG) along with low levels of high density lipoproteins (HDL - also known as "good cholesterol") are strongly associated with obesity, type 2 diabetes mellitus, and atherosclerosis and consequently the risk for CHD. HDL stands out from other lipid markers since it is known to have an active role in reducing the size and amount of atherosclerosis plaques, and plays a beneficial role in obesity, and obesity-related disorders, such as diabetes. Thus, by increasing
HDL levels one can not only delay cardiovascular disease progression but actually reverse its course.

[0004] The conventional methods for controlling abnormal lipid metabolism are through reduction of dietary intake of fats and treatment with cholesterol and obesity-reducing drugs. Drugs for diabetes have vanishing long-term effects on lipid metabolism.

[0005] LDL cholesterol is the main, if not the only, lipid target in the effort to reduce CVD morbidity and mortality. Clinical and epidemiological studies have identified HDL as a more promising target independently and inversely associated with an increased risk of CHD. LDL-lowering drugs, such as niacin, fibrates, and statins, are not very effective in raising HDL. The meta-analysis of clinical trials has shown that average HDL elevation in statin trials was 1.6 mg/dL, fibrate trials 2.6 mg/dL, and combinations trials of statins with niacin 12 mg/dL. In terms of %age, statins, fibrates, and nicotinic acid, increase HDL by 5-10%, 10%, and 20% respectively.

[0006] Currently approved anti-obesity drugs such as orlistat, sibutramine, and rimonabant, show only limited efficacy and are often associated with unpleasant side-effects, which account for a high attrition rate. The meta-analysis of data from obesity drug trials, which included waist circumference (WC) as an endpoint, reveals that orlistat therapy reduced WC by 2.1 cm (95% CI 1.3-2.9); sibutramine by 4 cm (95% CI 3.3-4.7); and rimonabant by 3.9 cm (95% CI 3.3-4.5 cm) (Padwal R, Li SK, Lau DC. Long-term pharmacotherapy for obesity and overweight. Cochrane Database Syst Rev 2003;4:CD004094).

[0007] The extent of the beneficiary effect of diet is limited, and drugs for cholesterol, diabetes, and obesity are often associated with unwanted side effects. Compliance is a significant concern. For example, less than 50% of patients continue to take statins after one year. Less than 10% of patients remain on obesity drugs after one year. Such high attrition rates undermine the efficacy of currently available drugs. Thus, there remains a need for a safer and effective means to prevent and/or treat atherosclerotic and metabolic.
Earlier on, atherosclerosis and obesity were merely viewed as lipid-storage diseases, but it is increasingly clear that low-grade inflammation is the underlying cause for these conditions. The chronic inflammation is caused by an autoimmune reaction against self-antigens and therefore this immune imbalance needs to be corrected in order to overcome obesity and atherosclerosis.

It is now generally acknowledged that atherosclerosis is an inflammatory disease - a concept which has been around since 19th century. Recent studies published during the last decade have suggested that obesity is also a chronic inflammation that is caused by a self-directed immune reaction against adipose tissue. However, the anti-inflammatory drugs are not effective to treat these diseases. Thus, more specific modulation of the inflammatory response through vaccination, either prophylactic or therapeutic, may represent a valuable strategy to prevent and/or treat both atherosclerosis and obesity.

Fifty years ago Gero et al., published the first evidence of atheroprotective immunity, which was produced by vaccination with beta-lipoprotein - the main protein in LDL particles. (Rittershaus et al, Atherosclerosis 2003, 169:1 13-20). However, this breakthrough has been met with skepticism since other scientists, particularly Bailey et al., (Nature 1964; 201 :407-8), Arnold et al., (J Atheroscler Res 1961 ;1:240-6) and Siegler et al., (Lancet 1961 :277:403) failed to reproduce the findings of the Hungarian investigators.

Twenty years later, in the 1970's, Russian and Czech investigators have shown the atheroprotective effect in a series of immunization studies in which they used beta- and pre-beta-lipoproteins, cholesterol, very low density lipoproteins (VLDL), gamma-globulin, albumin, and even Candida albicans. However they were not able to show that LDL could be used as a candidate for vaccine - a fact indicating that not every antigen involved in lipid metabolism is necessarily atheroprotective. Despite success with several antigens, no vaccine effective in humans has been produced.

After another two decades of inactivity, a sudden surge of interest became apparent in 1990's when several groups in the USA and Western Europe have published the
potential role of cholesterol, LDL, oxidized form of LDL, beta 2-glycoprotein, heat-shock protein 65 (HSP-65), and avian herpesvirus as vaccine antigens that could possibly abrogate the atherosclerosis. Again while studies in animals were promising, not a single commercial vaccine has resulted from these efforts.

More recent studies published during this decade continued the investigation of earlier identified antigens. However, at the same time a shift toward new targets became apparent, perhaps, due to lack of progress with prior antigens. These included a wide variety of immunogens such as cholesteryl ester transfer protein (CETP), tumor necrosis factor alpha (TNF-a), IL-12, IL-2, HSP-60, apolipoprotein B, vascular endothelial growth factor receptor 2 (VEGF), angiopoietin-2 receptor (TIE2), CD99, phosphorylcholine, and Streptococcus pneumoniae.

The efforts to develop an obesity vaccine are of more recent history dating back to 2006. There are three published studies that have shown the potential of ghrelin and gastric inhibitory polypeptide (GIP) as candidate antigens for obesity vaccine. Again all these studies were conducted in animal models and it is not obvious whether these candidates will become a safe and effective vaccine. Although experimental vaccines against type 1 diabetes are being tested, no specific vaccine against type 2 diabetes is available that is under development.

After 50 years of studies in animal models, the overwhelming majority of which were reportedly successful, only one vaccine progressed into clinical trials. The first human vaccine trial was reported in 2003 by AVANT Immunotherapeutics. This vaccine (CETi-1) had as an immunogen a peptide from cholesteryl ester transfer protein (CETP) - the initial premise was that the inhibition of CETP activity would increase "good cholesterol" HDL concentrations. US Patents Nos. 7,078,036; 7,074,407; 6,555,113; and 6,410,022 are incorporated by way of reference. Indeed in rabbit studies the vaccine produced a 42% increase in HDL and reduced LDL by 24% (Rittershaus et al., Arterioscler Thromb Vase Biol 2000; 20:2106-12). Based on such encouraging results this vaccine has been moved into human trials. A few years later, the phase 2 trial revealed that while CETi-1 was well
tolerated and anti-CETP antibodies were induced in patients, HDL levels increased only marginally (6%) (Rittershaus, 2007). Thus, further development of the original formulation of CETi-1 as anti-atherosclerosis vaccine has been abandoned. This shows that developing commercially viable vaccine is not an easy task and selecting an antigen that can produce meaningful results, especially in humans, is not as obvious as it may seem even to those skilled in the art.

[0016] It is well known from many other endeavors in pharmacology that successes in animals do not automatically translate into a successful product that can be safely and effectively used in humans. As a rule, the vaccines that initially produced promising results in animals fail when they are tested in humans. As seen supra this rule is equally applicable to the vaccines in the field of instant invention. Even more difficult is to make an orally effective vaccine. In view of such difficulties facing the development of vaccines for obesity, diabetes and atherosclerosis it is clear that traditional reductionist approaches are not very successful. Despite the fact that the first vaccine pertinent to this field of invention has been successfully tested in animals 50 years ago, until today there is no commercially available vaccine on the market. Therefore, obvious approaches have not succeeded and it would require a conceptual leap in the current thinking in order to overcome the existing gap between animal models and humans.

SUMMARY OF THE INVENTION

[0017] The present invention concerns a surprising discovery that an oral composition originating from adipose tissue (fat) or ultimately from adipocytes (fat cells) produces a desirable clinical effect in humans that is safer and better than the effect produced by currently available cholesterol, diabetes and obesity drug treatments.

[0018] It is contemplated that an increase in levels of HDL and a decrease in fat deposit indices, as well as reduction of insulin resistance, is due to modulation of the immune system as afforded by vaccines and immunomodulators. There is no precedent in the prior art that a single oral vaccine can effect simultaneously three independent parameters of metabolic disorders such as atherosclerosis, obesity and diabetes.
According to one aspect of the present invention a composition is provided, which is an oral composition originating from adipose tissue or in another words derived from an adipocyte, and which comprises or consists of water-insoluble, hydrolyzed proteins and fragments thereof embedded in a metal salt carrier. The metal is preferentially from alkaline earth metals. The terms adipose tissue and adipocytes have an equivalent meaning, since collectively the adipocyte cells are building blocks of the adipose tissue - a term known to laypersons as white fat or brown fat. Technically and for purposes of this invention, the term fat is not synonymous with adipose tissue or adipocyte—fat means to those skilled in the art and as used herein as a group of lipids known as triglycerides. Adipocytes are not necessarily always derived from fat or adipose tissue - they are obtainable from other biological tissues where they are present or from sources such as cultures of primary or immortalized adipocyte cell lines.

In another aspect of the invention infectious agents, such as microorganisms like bacteria, fungi or viruses, which are shown or suspected to cause metabolic disorders are employed as an additional antigenic material to be admixed to the instant composition.

The present invention also comprises a method of treating or preventing atherosclerosis, obesity and/or an obesity related disorders. Examples of obesity related diseases or disorders include but are not limited to overweight, metabolic syndrome, dyslipidemia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, lipodystrophy, steatosis or fatty liver, stroke, myocardial infarction, hypertension, gallstones, Alzheimer disease, arthritis, ulcerative colitis, lupus erythematosus, and even some forms of cancer. The present invention also provides a method of reducing blood pressure in an obese mammal.

The present invention also provides a process of making compositions of the present invention. In certain embodiments, the process comprises hydrolyzing adipose tissue or adipocytes, removing the lipid fraction such as free lipids and lipid-containing particles and collecting hydrolyzed proteins and fragments thereof, binding or embedding in a metal carrier, such as alkaline earth metal salt, treating with heat at temperatures higher than those
conventionally considered as denaturing temperatures, and formulating the resulting active ingredient as an oral tablet or pill. More specifically pharmacologically active compositions of the present invention are prepared by a process comprising: step (a) which consists essentially of hydrolyzing the adipose tissue or adipocytes in such a manner that lipid content is reduced and protein fraction is enriched. The hydrolysis is affected by art-known hydrolyzants, such as an acid or a base, of which the preferred hydrolyzant is an acid and the pH is below 3. In the case of a base hydrolyzant the pH is above 8. The duration of hydrolysis is less than 6 hours, and preferably less than 5 hours. In a preferred embodiment the composition obtained after step (a) will have more than of 25% protein but less than 8% free and 35% esterified cholesterol, less than 22% phospholipids and 10% triglycerides (by weight). The fatty acids, if any, are less than those found in the total plasma lipids and in lipoproteins. Preferably, the instant composition after step (a) processing of such sources ought to have less than 40% lipids, but protein fraction higher than 60% with lipid content being less than 1% in the final oral formulation. The protein fraction is composed of intact proteins and fragments thereof, such as polypeptides and peptides, so that at least one peptide remains that is responsible for the biological activity of the composition of the present invention. The composition contains at least one peptide from the protein fraction and preferably contains more than one peptide so the subject can mount an immune response.

[0023] In step (b) the precipitation of the active ingredient to a metal carrier or vehicle is undertaken. The example of preferred metal vehicle is magnesium or other alkaline earth metals like calcium, zinc, etc. These can be used in combination, for example magnesium to calcium ratio can be 2:1 or 3:1 ratio. The step (b) means that the protein fraction and derivatives thereof from the prior steps are bound or embedded within a carrier or vehicle. The term embedded as used hereinafter encompasses not only spatial relationship but also binding between metal and protein residues as a result of chemical reaction. In this way the pooled protein fraction is transformed following step (b) to a new chemical entity that is not found in the nature or can be obtained by simple traditional procedures such as cooking or rendering fat.
[0024] In step (c) the composition is heat-denatured; this step may be carried out after or simultaneously with step (b). The preferred temperature is above 80 °C, preferably higher than 120 °C for a period of time longer than 5 hours while assuring that the instant composition resulting from the hydrolysis is not surrounded by the aqueous medium. When step (c) is simultaneous with step (a) the aqueous medium can be present.

[0025] While steps (b) and (c) can be in any sequential order or can be carried out simultaneously with step (a), the final step (d), which is the formulation process that involves preparing an oral composition suitable for administration to a subject, such as a human, is always the last step in the process.

[0026] The preferred formulation is a tablet, granule, lozenge, capsule, dragee, nonpareils, comfits, suppository, or pill containing in addition to the active ingredient other ingredients or excipients that are well known in the art. The final oral formulation such as a pill will have no more than 90 % but not less than 0.1 %, more preferably no more than 60 % but not less than 1 % of the active protenaceous ingredient (by weight) resulting from step (c). In the tablet the most preferred range is between 1 and 20 % of the active ingredient (by weight) resulting from step (c). An ideal oral composition to be administered to a subject will have between 3 and 15 % of the active ingredient (by weight) resulting from step (c).

[0027] In yet another aspect, compositions of the present invention do not have an adjuvant or co-stimulatory molecules such as commonly found in vaccines to enhance humoral or cell-mediated immunity. Nor does it have an antacid to counteract the acidic milieu in the stomach.

[0028] The present invention also provides use of the compositions of the present invention as a therapeutic or prophylactic vaccine. The carrier or vehicle-linked protein composition will have activity comparable to drugs designed for treatment of atherosclerosis, obesity and type 2 diabetes. Although mostly contemplated as a therapeutic modality, the compound of this invention is also useful in preventing or reducing risk of occurrence of said conditions.
The compounds of this invention are used for therapeutic or prophylactic purposes by formulating them with appropriate pharmaceutical carrier materials and administering an effective amount to a host in need thereof, such as a human or other animals in need thereof. The composition is also contemplated to be used in combination with existing and yet to be marketed pharmaceutical drugs prescribed for treatment of atherosclerosis, obesity and type 2 diabetes.

The method is effected by orally administering to a subject in need thereof a therapeutically effective amount of a composition of the present invention thereby treating or preventing obesity or the obesity related disease in said subject.

Numerous additional aspects and advantages of the present invention will become apparent upon consideration of the figures and detailed description of the invention.

BRIEF DESCRIPTION OF THE FIGURES

The advantages of instant invention are disclosed herein by way of example only. In this regard, no attempt is made to show the invention in more detail than is necessary for the fundamental understanding of the invention, the detailed description together with the drawings below are explicit to those skilled in the art as to how several embodiments of the instant invention may be implemented in practice.

Figure 1. Changes in total plasma cholesterol (CH; -0.8%; p=0.75)(Figure 1A), low density lipoproteins (LDL; +3%; p=0.18)(Figure 1B), triglycerides (TG; -26.1%; p=0.29)(Figure 1C), and high density lipoproteins (HDL; +25.9%; p=0.000002)(Figure 1D), resulting from oral administration of a composition of the present invention as evaluated by repeated measure ANOVA. Individual values from each of 13 patients, collected through weeks 2, 4, 8 and 12, are plotted and mean values are shown in each graph in bold.

Figure 2. The effect of daily dose of the instant composition on TG/HDL ratio (-44.7%; p=0.12)(Figure 2A), waist (-7.6%; p=0.002)(Figure 2B), mid-arm (-3.3%;
p=0.049)(Figure 2C), and thigh (-7.6%; p=0.0003)(Figure 2D) circumferences as followed through weeks 2, 4, 8 and 12 and evaluated by repeated measure ANOVA. Individual values from each time-point for every patient are plotted and mean values are shown as bold line.

**DETAILED DESCRIPTION OF THE INVENTION**

[0035] The instant invention relates to a composition of matter, process of making and using compositions of the present invention. In particular methods are disclosed of treating or preventing atherosclerosis, obesity and related diseases via administering the composition to a subject in need thereof.

[0036] The principles and operation of the methods, uses and process of manufacture according to the present invention, may be better understood with reference to the drawings and detailed description hereinafter.

[0037] Compositions of the present invention may be prepared as described below. These examples comprise preferred embodiments of the invention and are illustrative rather than limiting.

[0038] Proteins from pooled adipocytes: An ordinary fat cell (also known as an adipocyte or lipocyte) contains a large lipid droplet surrounded by a layer of cell membrane. The nucleus is flattened and located on the periphery. A typical fat cell is about 0.1 mm in diameter with some being twice that size and others half that size. The lipid is in a semi-liquid state, and is composed primarily of triglycerides and cholesteryl ester. The adipose tissue and hence adipocytes can be obtained from easily accessible primary sources such as animal fat, eggs, whole blood and milk, and other art-known sources such as cell cultures in which adipocytes are present. In certain embodiments, a preferred source however is adipose tissue. It is generally understood that animal fat is obtained from the tissues of mammals and/or poultry. When fat tissues are derived from more than one animal they are pooled and subsequently proteins derived from them are also termed pooled proteins. Fat can be white fat or brown fat. Fat can be visceral fat or abdominal fat, including mesenteric, epididymal white adipose tissue (EWAT) and perirenal depots, subcutaneous fat including panniculus adiposus,
fat from liposuction or abdominoplasty procedures, areolar connective tissue, intramuscular fat, etc. Fat and lipids do not have the same meaning for the purposes of this invention. Human fat tissue for example contains about 87% lipids. The term lipid as used herein consists of fatty acids, e.g., oleic acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, and fatty acid derivatives (including tri-, di-, and monoglycerides and phospholipids), cholesterol, and phosphatidylcholine. In another classification, which is based on two distinct types of biochemical subunits - ketoacyl and isoprene groups - lipids may be divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids and polyketides. Regardless of their classification, these lipids are outside of scope of this invention, as compositions of the present invention encompass primarily hydrolyzed proteins and fragments thereof. Free lipoproteins are another source of material that can be transformed according to the principles and spirit of the compositions of the present invention. Particles comprising high density lipoproteins (HDL), low density lipoproteins (LDL) or very low density lipoproteins (VLDL), or a combination thereof, can be used as well as long as they are not in particulate form.

[0039] The composition resulting from hydrolysis step will have more than of 25 per cent protein but less than 8% free and 35% esterified cholesterol, less than 22% phospholipids and 10% triglycerides (by weight). The fatty acids content, if any, will be less than those found in the total plasma lipids and in lipoproteins. Preferably, the composition of the invention after initial processing of fat sources should have less than 60% lipids, but have a protein fraction higher than 40%. As the present invention contemplates proteins, the lipid content should be even a smaller portion in the final formulation. In a preferred embodiment the protein will have only traces of lipids, usually less than 10%, more preferably less than 5%, with preferred content under about 1% of free lipids and free from lipid particles.

[0040] The optimal content of proteins is assured by separating non-miscible lipid from the heavier density protein fraction, either by simply allowing the mixture to stand still until two clear-cut layers are formed in the suspension, or by centrifugation, or by filtration, or by a combination of these procedures. The protein fraction is composed of proteins and fragments thereof such as polypeptides and peptides so that at least one peptide remains that is
responsible for the biological activity of the composition of interest. However, it is more preferable that instead of just one protein or fragment thereof, a plurality of proteins and fragments is present in the composition. It is more preferable that this plurality of proteins and fragments is also pooled, i.e., obtained from pooled adipose tissue or pooled adipocytes.

[0041] The present invention is diametrically opposite to the reductionist approaches in the prior art, which rely on a single antigen, such as for example CETP. Despite failure with CETP all current experimental investigations aim at finding a solution by using a single target. Examples of such efforts are illustrated by allowed US Patent Nos. 7,527,795; 7,544,360; 7,078,036; 6,664,230; 6,410,022; 5,851,996; and 5,424,068. Occasionally, two targets are claimed to compose a vaccine such as for example in US Patent No. 5,753,260.

[0042] The prior art strategy is understandable, since a vaccine that has more than one purified antigen is prone to cause unpredictable adverse effects due to a cross reaction with unrelated host proteins. Even use of a single antigen can cause unpredictable reactions, especially when a vaccine candidate is moved from animals to a human trial. The present invention is thus novel and not obvious to those skilled in the art.

[0043] It is to be understood that term protein comprises ordinary proteins without prosthetic group and so-called conjugated proteins such as lipoproteins, lipophorins, apolipoproteins, glycoproteins, phosphoproteins, hemoproteins, flavoproteins, metalloproteins, phytochromes, cytochromes and opsins as long as they contribute to abnormal lipid metabolism. The term protein fragments refers to polypeptides and peptides as short as three to two amino acids length derived from a protein of interest.

[0044] Adipocyte culture as a source: Primary adipocytes are isolated from adipose tissue of piglets by mincing and digesting with collagenase at 37 °C for 2 h. The primary adipose cells are then washed extensively and incubated at 37 °C in Dulbecco's modified Eagle's medium containing 10 mM Hepes, 2% fetal calf serum, 1% bovine serum, albumin, penicillin, and streptomycin. Optionally, lipoblasts are stimulated with insulin and/or glucose to promote adipocyte growth and maturation. Adipocytes can be also derived from primary
cell culture such as pre-adipocyte stem cells, mature adipocytes, monocytes that are capable to differentiate into adipocytes, or simply by harvesting continuously replicating mature adipocyte or immature pre-adipocyte cell lines of mesenchymal origin. There are several such lines are available, e.g., obi 7, 3T3-L1, 3T3-F442A, ScAP-23, AP-18, Chub-S7, LS-14, PAZ6, SGBS, SW872, and hASCs, which are originally derived from adipose tissue of various animals as well as humans. Lipomatous benign or malignant sources of adipocytes such as a liposarcoma, myelolipoma, myxoid liposarcoma, lipoma, angioliopoma, angiomyolipoma, angiolipoleiomyoma, pleomorphic lipoma, neural fibrolipoma, chondroid lipoma, spindle-cell lipoma, intradermal spindle cell lipoma, hibernoma, are equally advantageous either as a primary source to be used directly or as a source for expanding in cell culture. After expansion either in adhering-monolayer or ceiling-floating type cultures to the desired quantity, the adipocytes are collected, flash-frozen in liquid nitrogen, and stored at -80 °C until use as a starting material compositions of the present invention. Alternatively, prior to freezing step a preparation of fat cell "ghosts" can be obtained in which lipids are removed. A standard art-known procedure consists of suspending intact cells in a hypotonic medium. Fat cells are broken by repeated exposure of intact cells to the hypotonic medium, centrifugation at 900 g, and by collection of the turbid aqueous phase. Then 40% sucrose (w/v) is added to make a final sucrose concentration of 8.5%. The aqueous suspension, containing the ghost cells, is subjected to a series of centrifugations and the resultant sediment is suspended in sufficient ice-cold bicarbonate buffer to give approximately 1 mg of ghost protein/ml, which can be used for starting steps of making compositions of the present invention.

[0045] Infectious pathogens as causative agents: The present invention is also based on the fact that certain microbial agents and viruses can cause or are associated strongly with atherosclerosis, obesity and diabetes. Examples of obesogenic viruses include, but are not limited to, canine distemper virus, Rous-associated virus type 7, Borna disease virus, scrapie agent, human adenovirus Ad-36, Ad-37, and Ad-5, animal adenovirus SMAM-1, and hepatitis virus. Infectious agents associated or causing atherosclerosis include Streptococcus pneumoniae, Chlamydia pneumoniae, Candida albicans, herpesvirus, Epstein-Bar virus, influenza virus, HIV, and chick embryo lethal orphan virus (CELO virus). See for example
US Patent No. 6,471,965, which describes use of herpesviruses to prevent development of atherosclerotic plaque. This composition however does not include adipocyte-derived material. The origin of diabetes mellitus was linked to a number of pathogens including, but not limited to, Helicobacter pylori, coxsackievirus, foot-and-mouth disease virus, enterovirus B, encephalomyocarditis virus, herpesviridae, cytomegalovirus, reoviridae, retroviridae, Venezuelan equine encephalitis virus, arbovirus, chickenpox, mumps and rubella viruses. Often these viruses and microorganisms are implicated in other metabolic syndromes and even in obesity-related cancer. These agents can be propagated in infection-susceptible adipocytes or other convenient substrate cells like epithelial or lymphoid cells in vitro or in vivo such as in embryonated chicken eggs that are then hydrolyzed, heat treated and served as a lipid-free source to be added to the compositions of the present invention, either in purified form or along with the cell substrate in which they are propagated. Molecular means of virus expansion such as cloning of virus or their antigenic parts are also contemplated by art-established techniques. Alternative art-known means of creating antigens from infectious agents, like VLP or virus-like-particle or artificial synthesis of suitable antigens are equally suitable. In a preferred embodiment these pooled microbial or viral antigens are mixed with the instant adipocyte-derived composition at therapeutically effective ratio such as for example 1:1-100 or any other ratio that can be determined without undue experimentation. Microorganisms, which for purposes of this invention comprise bacteria, fungi and viruses, are required to be heat killed or sterilized either before or after mixing with the instant composition. Dry heat is more advantageous for the purposes of this invention and is equally applied to microorganisms as well as for the instant composition prior to the oral formulation step. The prior art standard setting for a hot air oven is two hours at 160 °C. A rapid method heats air to 190 °C for 6-12 minutes. The preferred dry-heating step in the present invention is at least 2-fold longer than in the prior art heating conditions.

[0046] Process steps for manufacturing compositions of the present invention consist of step (a) protein hydrolysis; step (b) precipitation of hydrolyzed proteins; step (c) dry-heating hydrolyzed proteins; and step (d) formulating into an oral formulation. These steps are not necessarily carried out in sequential order; further in some embodiments some steps such as (a) through (c) can be simultaneous as some of foregoing examples show. Processing steps
(a), (b), (c), and (d) for manufacturing the instant composition include but are not limited to the following examples.

[0047] A typical fat or adipose tissue consists of about 85% lipids and 15% proteins. Most of the proteins of interest are located within the cell membrane. The lipids are less dense than water and are insoluble in aqueous solution and tend to float on the surface of aqueous solution, whereas proteins are more dense and soluble in water and form a distinct fraction found below lipid layer. Due to this difference they form two distinct fractions when they are suspended in an aqueous medium. For the purposes of this invention proteins rendered insoluble in water is the fraction of interest. Free lipids and lipid particles comprising high density lipoprotein (HDL), low density lipoprotein (LDL) or very low density lipoprotein (VLDL) particles, or a combination thereof are in the lipid layer. The process of hydrolysis not only facilitates the physical separation of lipid and protein, but also breaks down proteins to smaller fragments such as polypeptides and peptides. By controlling conditions of the hydrolysis reaction one can obtain essentially purified proteins and fragments thereof in which very small amount of free lipids may be present, and which is is devoid of lipid particles.

[0048] The hydrolysis step starts with physical fragmenting of adipose tissue and subjecting the obtained slurry to a hydrolyzant such as, for example, hydrochloric acid at pH below 3, and for a duration of time whereby the protein fraction is hydrolyzed and the lipid fraction remains in the same continuously stirred suspension. Hydrolysis can be achieved by exposing to a pH higher than neutral such as by exposing to a base. However, acidic hydrolysis is more preferable. Depending on the pH, i.e., the acid concentration, the protein fraction is hydrolyzed in a dose-dependent manner to smaller fragments such as polypeptides and peptides. The duration of the process of the present invention may last from between about 0.5 h to about 10 h, preferably less than 5 h. The respective proportions of source material and hydrolyzant can vary widely depending on the desired degree of hydrolysis. One of ordinary skill in the art can determine with little or no experimentation the desired stoichiometric ratio of hydrolyzant and raw material to be treated. Preferably, the mixing proportions include a ratio of between about 1:1 to about 1:30 and more preferably from
between about 1:1 to about 1:10 by volume. The hydrolyzed protein fraction is then separated from the lipid fraction by gravity, centrifugation or filtering. The precipitation of proteins to form insoluble protein facilitates collection of protein fraction as described in detail hereinafter.

[0049] Precipitation is a widely used process in downstream processing of biological products, such as proteins. The underlying mechanism of precipitation is to alter the solvation potential of the solvent and thus lower the solubility of the solute by addition of a reagent.

[0050] This phenomenon, often termed "isoelectric point precipitation," is well known even to laypersons as illustrated by the protein coagulating effect of lemon juice when it is added to a glass of milk. The acidification with organic acids or inorganic acids are equally advantageous. This phenomenon depends on the isoelectric point (pi) which is the pH of a solution at which the net primary charge of a protein becomes zero. The pi of most proteins is in the pH range of 4-6. Examples of organic acid are acetic acid, abietic acid, carboxylic acid, rosin, ethoxybenzoic acid, polyalkenoic acid, polyacrylic acid, and tannic acid. Mineral acids, such as hydrochloric and sulfuric acid may be used as precipitants. However, this procedure is considered disadvantageous to those skilled in the art since irreversible denaturation is caused by acids. Thus, the instant invention is contrary to this commonly held belief.

[0051] Another well known method called the "Cohn process" is commonly used for plasma protein fractionation such as albumin or gamma-globulin, and which relies on solvent precipitation with ethanol. Addition of miscible solvents such as ethanol or methanol to a solution may cause proteins in the solution to precipitate. Important parameters to consider are temperature, which should be less than 0 °C to avoid denaturation, pH and protein concentration in solution. Miscible organic solvents decrease the dielectric constant of water, which in effect allows proteins to come close together and precipitate. This method however is reliable only for certain proteins and does not encompass the totality of proteins and is validated for plasma or serum proteins not from adipocyte sources.
Another methods known as "flocculation by polyelectrolytes" such as with alginate, carboxymethylcellulose, polyacrylic acid, tannic acid and polyphosphates, which form extended networks between protein molecules in solution. The effectiveness of these polyelectrolytes depends on the pH of the solution. Anionic polyelectrolytes are used at pH values less than the isoelectric point. Cationic polyelectrolytes are at pH values above the pi. It is important to note that an excess of polyelectrolytes will cause the precipitate to dissolve back into the solution. An example of polyelectrolyte flocculation is the removal of protein cloud from beer by using Irish moss.

Yet, another method known in the art is precipitation with polyvalent metallic ions such as Ca2+, Mg2+, Mn2+, A13+ or Fe2+. This method can be regarded as a combination of steps (a) and (b) to be carried out simultaneously. Metal-containing acids react easily the water-miscible protein fraction and/or by heating the impregnating solution to provide better interaction of the metal salt with the tertiary amino nitrogen. The amount of metal cation salt to mix with proteins can be varied from 5% by weight to stoichiometric amounts based on amine content of the protein and is usually from 50% to 100% by weight thereof. In general, interaction of divalent or trivalent cations with proteins is enhanced at higher cation concentrations (1 to 20 mM) such that induction of major conformational transitions in native a-helix and β-sheet is achieved. In another embodiment, the concentration of salt is within the range of about 0.1 to about 4 M. By way of example, in a typical run, 1 gram of magnesium chloride is added for each 100 grams of solution of proteinaceous material and adjusted to the appropriate hydrolyzing pH (3.0 N HCl or 5.0 N NaOH). The final ratio is determined by stoichiometry of reaction and by monitoring levels of protein remaining in reaction solution. Samples of the supernatant are periodically withdrawn at the designated time interval for analysis of remaining soluble peptide content by a standard method such as Lowry method. When most of protein is precipitated, the content of soluble protein will be minimal. The content of total soluble protein remaining in the solution must be less than 70 mg/ml in order to satisfy optimal precipitation. Where appropriate, instead of aluminum or magnesium, the addition of calcium salts or other calcium containing ingredients such as calcite, calcium oxide, calcium phosphate, calcium carbonate, calcium aluminate or calcium silicate is also contemplated. This procedure is illustrated by the process of making
tofu from soy milk, which relies on use of calcium or magnesium salts or the use of acids such as glucono delta-lactone to precipitate protein mass.

[0054] The example of hydrolysis followed by precipitation is based on the fact that while protein salt such as formed by reaction with hydrochloric acid is very soluble in water, the base is almost insoluble. In order to precipitate soluble protein, two parts protein+HCL are mixed with one part baking soda. The solution is gently heated until white precipitates form.

[0055] The protein fraction can be obtained from various other chemical methods for precipitating proteins or peptides are known and have been described in the art. Salting out is the most common method used to precipitate a target protein. Addition of a salt, such as ammonium sulfate or sodium citrate, compresses the solvation layer and increases protein-protein interactions. As the salt concentration of a solution is increased, more of the bulk water becomes associated with the ions. As a result, less water is available to partake in the solvation layer around the protein, which exposes hydrophobic patches on the protein surface. Proteins may then exhibit hydrophobic interactions, aggregate and precipitate from solution.

[0056] The separation of lipids from proteins can be done by other art-known methods such as, for example, dextran sulfate, amylopectin sulfate, heparin or polyvinylpyrrolidone. Another method of separation is hydrophobic interaction chromatography using phenyl sepharose. However, a preferred method of separation is the use of metals such as magnesium, instead of above listed precipitants. In lieu of pure metal itself, a salt of the metal is more advantageous. An example of salt of a representative metal, such as for example magnesium, is aluminium magnesium boride, calcium magnesium acetate, dimagnesium phosphate, magaldrate, aluminde, aspartate, benzoate, bromide, carbonate, chloride, chloride hexahydrate, citrate, diboride, diglutamate, diuranate, fluoride, gluconate, hexahydrate, hydride, hydroxide, iodide, lactate, levulinate, nitrate, nitride, orotate, oxide, oxychloride, oxysulfate, perchlorate, peroxide, phosphate, pidolate, silicide, stearate, sulfate, sulfide, sulfite, trisilicate, monomagnesium phosphate, trimagnesium citrate and the like. Other metals can be used which have equivalent usefulness including but not limited to: lithium,
beryllium, sodium, silicone, aluminium, aluminum, potassium, calcium, titanium, vanadium, chromium, manganese, cobalt, nickel, copper, zinc, zirconium, molybdenum, silver, selenium, antimony, barium, platinum, gold, mercury, thallium or salts thereof. The preferred metals are iron, zinc, copper, cobalt, sodium, silicone, aluminium, aluminum, potassium, manganese, calcium and magnesium. In general, alkaline earth metals such as calcium and magnesium, as well as zinc and aluminum are more preferable over other metals. Other non-toxic metals and their salts include sodium and potassium, and salts thereof, such as sodium bicarbonate. Other forms of metals include, for example, zinc oxide eugenol, zinc stearate, zinc acetate, zinc phosphate, zinc polycarboxylate, glass ionomer, silico phosphate, etc. These salts of which magnesium and calcium salts are preferred, are metal carriers in which proteins and their fragments are embedded and together serve as an active ingredient in compositions of the present invention.

[0057] A preferred heating method is dry-heating. The precipitate in the form of protein cake embedded within metal carrier such as magnesium or calcium is washed with distilled water and dried for 24 hours under infrared lamp or baked in an oven or food dehydrator at 120 °C for about 5-10 hours or preferably more. The precipitate can be further washed with a solvent, such as ethanol, to further remove solubles, such as lipids. The variables in time or temperatures are not critical as long as they exceed conditions that were considered in the prior art as unacceptable, due to fact that proteins are irreversibly denatured or in other words rendered useless when they are heated at protein-denaturating temperatures above 57 °C for too long. Denaturation is conventionally regarded as a process in which proteins or nucleic acids are exposed to some external stress or compound, such as a strong acid or base, a concentrated inorganic salt, an organic solvent (e.g., alcohol or chloroform), or heat. This is why boiled eggs become hard, and cooked meat becomes colorless and firm. It is believed by those skilled in the art that if a protein is denatured it loses its potential to induce an immune response of a host to the native non-denatured protein.

[0058] Industrial scale reactors can be used to precipitate large amounts of proteins, and can be used in the present invention. Batch reactors are the simplest type of precipitation reactor. The precipitating agent is slowly added to the protein solution under mixing. The
aggregating protein particles tend to be compact and regular in shape. Since the particles are exposed to a wide range of shear stresses for a long period of time, they tend to be compact, dense and mechanically stable. In tubular reactors, feed protein solution and the precipitating reagent are contacted in a zone of efficient mixing then fed into long tubes where precipitation takes place. Turbulent flow is promoted through wire mesh inserts in the tube. The tubular reactor does not require moving mechanical parts and is inexpensive to build. However, the reactor can become impractically long if the aggregation is slow. In continuous stirred tank reactors, a continuous flow of reactants and raw material are in a well-mixed tank.

[0059] The dried powder of the active ingredient(s) (which contains peptides and protein fragments) obtained from steps (a) to (c) is then mixed with excipients and formulated as a tablet. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, talk, etc. Suitable carriers include lubricants and inert fillers such as lactose, sucrose, or cornstarch. In another embodiment, these compounds are tableted with conventional tablet bases such as lactose, sucrose, or cornstarch in combination with binders like acacia gum, cornstarch, or gelatin; disintegrating agents such as cornstarch, potato starch, or alginic acid; a lubricant like stearic acid or magnesium stearate; and sweetening agents such as sucrose, lactose, or saccharine; and flavoring agents such as peppermint oil or artificial flavorings.

[0060] A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and the filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier, for example aqueous gum, cellulose, silicates or oil and the dispersion or suspension then filled into a soft gelatin capsule. Typical parental compositions consists of a solution or suspension of the compound in a sterile aqueous carrier. Alternatively, the solution can be in powder form and then reconstituted with a suitable solvent just prior to administration. A typical suppository formulation comprises a compound with a binding and/or lubricating agent such
as polymeric glycols, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

[0061] Importantly the instant orally formulated vaccine contains no adjuvants, co-stimulatory molecules, nor antacids. In a preferred embodiment, compositions of the present invention, when it is to be delivered orally, does not require an adjuvant or other co-stimulatory molecules such as commonly found in vaccines that enhance classical humoral or cell-mediated immunity. Nor does it have an antacid to counteract the acidic milieu in the stomach.

[0062] Preferably the composition is in unit dose form such as a tablet or pill. Each dosage unit for oral administration contains preferably from 1 to 1000 mg, more preferably between 10 and 100 mg, of instant active ingredient or a pharmaceutically acceptable salt thereof.

[0063] The compound of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for example, an oral dose of between 1 and 5 tablets, preferably between 1 and 2 tablets, the compound being administered 1 to 4 times per day. The duration of treatment can be continuous or interrupted as prescribed by a physician.

[0064] It is also contemplated that instant composition is used in combination with therapeutically effective amount of an additional active agent or drug that is developed for obesity, atherosclerosis and diabetes indications. In addition the instant composition can be used in combination with a diet.

[0065] Obesity related diseases: Common examples of obesity related diseases or disorders include but are not limited to overweight, metabolic syndrome, dyslipidemia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, lipodystrophy, steatosis or fatty liver, stroke, myocardial infarction, hypertension, gallstones, Alzheimer disease, arthritis, ulcerative colitis, lupus erythematosus, and even some forms of cancer. Some of these
conditions occur in large part as a result of insulin resistance induced by obesity, giving rise to type 2 or non-insulin dependent diabetes mellitus.

[0066] Various anti-obesity drugs are known in the art and these include by way of example appetite suppressants, e.g., catecholamines and their derivatives such as amphetamine-based drugs (Benzedrine). Similar in action are dexfenfuramine (Redux), Fen-phen, and phenylpropanolamine. Drugs blocking the cannabinoid receptors such as Rimonabant (Acomplia) is another category of suppressants of the appetite. Sibutramine (Reductil or Meridia) which is a noradrenaline/serotonin reuptake blocker is an anorectic or appetite suppressant, reducing the desire to eat. Orlistat (also known as Xenical and Alli) is an example of a gastric lipase inhibitor. Other lipase inhibitors in development are GT 389-255 and Cetilistat. Exenatide (Byetta) is a long-acting analogue of the honnnone GLP-1, which the intestines secrete in response to the presence of food and promotes a feeling of satiety. Pramlintide (Symlin) is a synthetic analogue of the honnnone amylin, which promotes a feeling of satiety.

[0067] Statins or HMG-CoA reductase inhibitors are a major class of drugs that lower cholesterol levels, especially LDL. Several exist today including atorvastatin (Litor, Torvast); cerivastatin (Lipobay, Baycol), fluvastatin (Lescol); lovastatin (Mevacor, Altorcor, Altoprev); mevastatin; pitavastatin (Livalo, Pitava); pravastatin (Pravachol, Selektine, Lipostat); rosvastatin (Crestor); and simvastatin (Zocor, Lipex). Some of the statins are available in combination such as lovastatin+niacin (Advicor); aimvastatin+Ezetimibe (Vytorin); atorvastatin+amlodipine besylate (Caduet); and simvastatin+niacin (Simcor). Atherosclerosis drugs other than statins include niacin and derivatives thereof, also known as vitamin B3 or nicotinic acid; fibrates such as bezafibrate (Bezalip); ciprofibrate (Modalim); clofibrate; gemfibrozil (Lopid); and fenofibrate (TriCor); and bile acid sequestrants (resins) such as cholestyramine (Questran), colesevelam (Cholestagel, Welchol), and colestipol (Colestid).

[0068] Examples of diabetes hypoglycemic drugs contemplated for use in combination with instant composition include but are not limited to: insulin; insulin such as
neutral protamine Hagedorn (NPH); and glargine insuline; insulin secretagogues of sulfonylurea class such as tobutamide (Orinase), acetohexamide (Dymelor), tolatamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), and gliclazide (Diamicron); short-acting secretagogues belonging to meglitinides such as repaglinide (Prandin), nateglinide (Starlix); incretin secretagogues such as glucagon-like peptide-1 (GLP-1) and their agonists such as exenatide (Byetta), liraglutide, and taspoglutide; dipeptidyl peptidase-4 antagonists acting as GLP enhancers such as sitagliptin (Januvia), vildagliptin (Galvus), saxagliptin (Onglyza); gastric inhibitory peptide (GIP); biguanide enhancers of glucose uptake like metformin (Glucophage), phenformin (DBI) and buformin; glitazone drugs acting with peroxysome proliferator responsive elements of thiazolidinedione such as rosiglitazone (Avandia), pioglitazone (Actos), troglitazone (Rezulin); alpha-glucosidase inhibitors such as miglitol (Glyset) and acarbose (Precose/Glucobay); and amylin agonist such as pramlintide. It is also contemplated that compositions of the present invention can be used with yet to be approved drugs like enhancers of sensitivity of glucokinase; PPAR ligands like muraglitazar and tesaglitazar; sodium-dependent glucose transporter 1 inhibitors; fructose 1,6-bisphosphatase inhibitors. It is equally advantageous that compositions of the invention can be used along with alternative medicines of plant or animal origin as well as with various vitamins like vitamins C, E, and K2, and minerals like chromium and vanadium.

[0069] Examples of anti-inflammatory drugs include, but are not limited to, steroids, e.g., corticosteroids; non-steroidal anti-inflammatory drugs like propionic acid derivatives, acetic acid derivatives, enolic acid derivatives, fenamic acid derivatives and COX-2 inhibitors; immune anti-inflammatory agents and various herbs having anti-inflammatory properties.

EXAMPLES

[0070] Example 1: Human clinical study. The study involved 9 females and 4 males, all of Asian origin, aged between 22 and 79, with mean/median age 39.8/38 years. The study entry mean body mass index (BMI) was 26.3 kg/m² - reflective of higher than normal (25 kg/m²) content of body fat - and which places them in overweight category among Asians.
Mean waist circumference (WC) in males (102.5 cm) and females (88.7) was above abdominal obesity threshold 90 cm and 80 cm respectively. The baseline HDL cholesterol was 38.6 mg/dL which is below 40 mg/dL cut-off normal value. The triglyceride (TG) entry levels were above normal 150 mg/dL, i.e., 163 mg/dL. Total cholesterol plasma content was within 200 mg/dL upper limit and LDL content was also within normal range 62-130 mg/dL. Systolic and diastolic blood pressure values were normal, i.e., 115.6 and 77.1, respectively. Baseline fasting blood glucose content (94.8 mg/dL) was also normal. Briefly, except normal baseline blood pressure and glucose, the patients were at increased risk of CHD, since they had abnormal baseline BMI, WC, TG, and HDL. Patients consented to receive twice-daily dose of two pills and be subjected to routine laboratory and physical check-ups at 2, 4, 8, and 12 weeks intervals. All patients, except patient #13 who received the composition for one month due to late entry into study, have received treatment for three months. The peripheral blood samples were drawn and sent to a certified commercial laboratory for complete CBC and standard biochemistry tests including liver, kidney, glucose, and lipid profile. Mid-arm, abdominal, and thigh diameters were measured with a flexible, non-elastic measuring tape at baseline and after 2, 4, 8 and 12 weeks.

[0071] Statistical analysis: Obtained data from study patients analyzed at 0.5, 1, 2, and 3 month intervals has been analyzed using repeated measure ANOVA test (STATMOST, Dataxiom, Los Angeles, CA). Where appropriate, basic parametric and non-parametric tests were utilized for the assessment of data. The probability values for all tests were considered significant at p<0.05.

[0072] Example 2: Safety. None of the patients had reported any adverse effects attributed to treatment, most had noted better mood and quality of life. While subjective, these impressions are corroborated by objective results from lab analysis. Pre- and post-treatment blood pressure systolic and diastolic values were not affected significantly, i.e., 115.6 vs 121 (p=0.2) and 77.1 vs 83 (p=0.55). Liver enzymes, ALT and AST, were not influenced by instant composition, i.e., 28.4 vs 26.1 and 23.1 vs 24.4 with p values 0.3 and 0.73, respectively. Quite contrary, patient #4 who had elevated ALT and AST levels (96 IU and 44 IU) at baseline had experienced reduction to 56 IU and 31 IU at the end of follow-up - a sign
indicating beneficial effect on liver function. The composition had no adverse effect on kidney function. Creatinine levels appeared to decrease; 0.877 vs 0.842 mg/dL (p=0.02) while blood urea nitrogen (BUN) indices have shown a trend toward increase; 14.8 vs 16.1 mg/dL (p=0.03). While statistically significant, both values remained within normal ranges; 0.5-2.0 mg/dL and 9-23 mg/dL for creatinine and BUN, respectively. Fasting blood sugar levels also remained within the normal range (70-130 mg/dL) even though a small upward trend has been observed, i.e., 94.8 vs 98.8 (p=0.04).

[0073] Complete blood cell (CBC) analysis has been carried out at regular intervals to identify changes that could be attributed to instant method of therapy. Hemoglobin levels had changed slightly from 13.25 to 13.19 g/dL (p=0.0004), which, however, remained within normal range 12.1-17.2 g/dL. This reflected in increase of hemoglobin content per red blood cell (MCH) from 25.62 up to 26.42 picograms/cell (p=0.000003), but hemoglobin concentration relative to size of the cell (MCHC) has not changed appreciably, i.e., 32.77 vs 32.75 g/dL (p=0.2). Hematocrit and red blood cells count had changed slightly, but remained within normal range 40.7 to 40.2% (p=0.02) and 5.22 to 5.05 x 10^6 cells/mm^3 (p=0.002) respectively. The average red blood cell size (MCV) increased from 78.5 to 80.0 femtoliters (p=0.00009). The number of platelets has moved upward, from 2.471 to 2.921 x10^5 per mm^3 (p=0.009). The mean white blood cells count has not changed: 7.931 vs 7.283 cells/mm^3 (p=0.63). The % of leukocytes and neutrophils has not been affected by the therapy; 37.3% vs 41.3% (p=0.22) and 59.7% vs 56.5% (p=0.38). Although pro-inflammatory eosinophils were seen to decline from mean 4.0% down to 2.4% the significance was not attained (p=0.29), mainly due to the undetectable levels of such cells at certain time-points in 6 out 13 patients.

[0074] The effect was measured for changes in body weight and body mass index (BMI). No significant alterations in body weight were found, with average weight prior to and after treatment being 67.87 vs 66.58 kg (-1.9%; p=0.46). Similar, non-significant results were obtained with BMI, i.e., 26.25 vs 25.75 kg/m² (-1.9%; p=0.35) (Fig. 2). The anthropometric predictors of body fat such as mid-arm, abdomen and thigh circumferences were evaluated by repeated measure ANOVA (Fig. 2). Mid-arm circumference had decreased in 11 out 13 individuals by average 3.3% from mean 30.85 cm to 29.83 cm at the end of three months (-
1.02 cm; p=0.049; 95% CI 0.6 - 2.8 cm). Waistline decreased by 7.6% in 11 out 13 individuals from average 92.96 to 85.92 cm (-7.04 cm; p=0.002; 95% CI 2.0-9.3 cm). The waist circumference, when stratified to 9 women, declined by 7.5% from abdominal obesity defining level 88.72 cm down to 82.11 cm (-6.6 cm; p=0.005; 95% CI 1.9- 11.3 cm). The thigh circumference has been reduced by 7.6% in 12 out of 13 individuals, i.e., 56.15 at baseline vs 51.91 cm (-2.96 cm; p=0.0003; 95% CI 2.2-6.2 cm). The similarity in outcome from these three sites of fat deposition in the prevailing majority of patients indicates that this trend is consistent and statistically significant despite small sample size.

[0075] Example 3: Effect on lipid profile and insulin resistance predictor. The serum levels of lipids such as total cholesterol, LDL, HDL and triglycerides have been analyzed at 2 week, 1, 2, and 3 month intervals after first administered dose of composition (Fig. 1). The total cholesterol content has not changed from the baseline value; 193.4 vs 191.8 (p=0.75). LDL levels fluctuated slightly upward but results were not statistically significant, i.e., 113.8 vs 117.2 (p=0.18). The cholesterol to LDL ratio has not changed considerably, i.e., 1.86 vs 1.67 (p=0.31). TG levels have been reduced in 11 out 13 patients with average intra-group decrease by 26.1%, i.e., from 163 to 120.5 mg/dL (p=0.29), but due to high TG values in patient #4, the results were skewed and statistically non-significant. The use of repeated measure, non-parametric Friedman test has not produced better significance as p value (p=0.21) was still above 0.05 cut-off value. Nevertheless, quasi-linear regression analysis that considers the gap between baseline and end of study TG values from outliers (patients #4 and #6) and remaining patients produced p=0.0000005 with R-squared regression coefficient 0.7. Most significant change was seen with HDL, which increased by 25.9% from 38.6 to 48.5 mg/dL (p=0.000002) in 12 out 13 patients. The average/median absolute increase in HDL at the end of 3 months treatment was equal to 9.9/11 mg/dL (range 16-3 mg/dL; 95% CI 5.7 - 12.5 mg/dL). LDL/HDL ratio decreased by 19.7% from 3.04 vs 2.44 (p=0.03). This change reflected in decrease of cholesterol to HDL ratio by 25% from 5.17 to 3.98 (p=0.0009). The TG/HDL ratio, which is a predictor of insulin resistance and CHD risk, has been reduced by almost half (44.7%; p=0.12) from mean 4.68 (95% CI 1.3-8.0) to 2.59 (95% CI 1.4-3.7) as evaluated by paired Student t-test.
Example 4: Lack of non-specific magnesium effect. In this study the contributing non-specific effect of magnesium is ruled out since magnesium alone can marginally increase in HDL (4-1 1%), but reduces CH (6-23%), LDL (10-1 8%) and TG (10-42%). The instant composition has no effect on CH and LDL, but increases the levels of HDL by about three-fold maximum attained levels achieved by magnesium alone and decreases all measured obesity indices. Furthermore, magnesium supplements tend to reduce blood pressure systolic (SBP) and diastolic (DBP) values. According to the Cochrane study, which combined all published trials, participants receiving magnesium supplements as compared to control did not significantly reduce SBP (mean difference: -1.3 mmHg, 95% CI: -4.0 to 1.5), but did statistically significantly reduce DBP (mean difference: -2.2 mmHg, 95% CI: -3.4 to -0.9). In the present study, no significant effect on blood pressure is found; contrary systolic and diastolic values have actually shown slight trend toward increase, i.e., SBP: 115.6 vs 121 mmHg (+5.4 mmHg; p=0.2) and DBP: 77.1 vs 83 mmHg (+5.9 mmHg; p=0.55). Finally, doses of supplemental magnesium that were reported to have significant clinical effect in the prior art are much higher than typical magnesium daily dose (360 mg) received in this study. For example, in the Rodriguez-Moran 2003 (Diabetes Care 2003;26:1 147-52) study showing improvement in insulin sensitivity, the daily dose of magnesium was about seven-fold higher, i.e., 2.5 gram, than in this study. Other studies typically relied on ten-fold or higher doses of magnesium. It is clear that the composition of the present invention taken at such a small dose of magnesium carrier will be of no effect on insulin resistance, yet we have dramatic effect in reducing TG/HDL ratio. Furthermore, Rodriguez-Moran study has not shown any effect on waist circumference, while in our study this predictor of obesity surpasses the best results seen to date in obesity drug trials. Taken together these data indicate unequivocally that the instant composition does not owe its beneficial effect to non-specific magnesium supplementation.

Example 5: Preventive use. Long term, follow-up studies revealed that incremental increase in absolute or percentage HDL values can reduce the risk of occurrence of metabolic diseases. The data from cholesterol-reducing drug trials indicated that for every 1% increase in HDL there was a 3% reduction in death or myocardial infarction. In other studies, per each 5 mg/dL increment in HDL there was a 29% risk reduction. If these figures
are extrapolated to our findings then the risk reduction due to preventive intervention by instant composition is between 40% and 89% - a benefit that surpasses the average 30% benefit associated with optimal LDL control with statins and the like. Indeed, none of the treated patients who were in a high risk group at study entry have suffered from dyslipidemia, hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, lipodystrophy, steatosis or fatty liver, stroke, myocardial infarction, hypertension, gallstones, Alzheimer disease, arthritis, ulcerative colitis, lupus erythematosus, or cancer. Furthermore, they did not gain excess weight, nor had any evidence of diabetes.

Example 6: Use for high blood pressure. Seven patients, six females and one male, aged between 47-83 years old, with mean±SD/ median 62.3±1.3/6/-56 with an obesity problem and who had high blood pressure refractory to common blood pressure drugs have accepted to try the instant composition. In addition to weight loss, the patients experienced decreased blood pressure which was statistically significant despite small sample size. This has been surprising, since the composition was intended to reduce excess weight and correct metabolic cholesterol disorder. Nevertheless, the instant composition taken at a dose of one pill per day, with or without blood pressure medicines, helped to essentially normalize excessive hypertension. In two patients, arrhythmia and tachycardia were corrected as well. These results indicate that present composition in addition to atherosclerosis and obesity indications is useful in correcting cardiac problems as well.

Table 1. Effect of daily dose of instant composition on high blood pressure.

<table>
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<th>No.</th>
<th>Patient's initials</th>
<th>Age</th>
<th>Sex</th>
<th>Upper Before</th>
<th>Upper After</th>
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<td>230</td>
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</table>

62.3 ±13.8 /56 6/1 194.3±20.7 /190 142.9±18.9 /140 117.5±33 /115 87.1±13.8 /90

P=0.0002 P=0.085
[0080] It is to be understood by those skilled in the art that the foregoing description and examples are illustrative of practicing the present invention, but are in no way limiting. Variations of the detail presented herein may be made without departing from the spirit and scope of the present invention as defined by the following claims.
CLAIMS

1. A composition comprising pooled adipocyte-derived proteins and fragments thereof embedded within a metal salt carrier and suitable for treatment or prevention of atherosclerosis, obesity, or obesity related disorders.

2. The composition of claim 1, wherein said metal salt is a magnesium or calcium salt.

3. The composition of claim 1, wherein said pooled adipocyte-derived proteins and fragments thereof are hydrolyzed.

4. The composition of claim 1, wherein said pooled adipocyte-derived proteins and fragments thereof are dry-heated.

5. The composition of claim 1, further comprising a killed microorganism associated with the atherosclerosis, obesity, or obesity related disorders is present in a hydrolyzed, lipid-free state.

6. The composition of claim 1, wherein said adipocyte is derived from an adipose tissue or adipocyte cell culture.

7. The composition of claim 7, wherein said adipose tissue is fat, whole blood, whole milk or egg.

8. The composition of claim 1, wherein said composition is formulated as a tablet or pill.

9. The composition of claim 1, wherein said composition is an oral vaccine.

10. The composition of claim 1, wherein said oral vaccine is without an adjuvant, a co-stimulatory molecule, or an antacid.

11. A method of treating or reducing risk of occurrence of atherosclerosis, obesity, or obesity related disorders, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of adipocyte-derived, hydrolyzed proteins and fragments thereof within a magnesium carrier.

12. The method of claim 11, wherein said subject is a human.

13. The method of claim 11, wherein said obesity related disorder is selected from the group consisting of overweight, metabolic syndrome, type 2 diabetes, dyslipidemia,
hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, lipodystrophy, steatosis, stroke, myocardial infarction, hypertension, gallstones, Alzheimer disease, arthritis, ulcerative colitis, lupus erythematosus, or cancer.

14. The method of claim 11, further comprising administering to the subject a therapeutically effective amount of an additional active agent.

15. The method of claim 14, wherein the additional active agent is selected from the group consisting of an appetite suppressant, a cholesterol absorption inhibitor, a cholesterol generation inhibitor, a gastric lipase inhibitor, a fat generation inhibitor, an anti-diabetic agent, a noradrenaline-serotonin reuptake blocker, a cannabinoid receptor antagonist, and an anti-inflammatory agent.

16. A method of making HDL-increasing and obesity or diabetes indices-reducing oral vaccine, to be used in treatment or for reducing risk of occurrence of atherosclerosis, obesity and obesity related disorders, the method comprising the steps of:

(a) hydrolyzing pooled adipocyte proteins;
(b) precipitating hydrolyzed proteins with a metal salt;
(c) heating hydrolyzed proteins;
(d) formulating the hydrolyzed, metal-embedded, heated proteins and fragments thereof into an oral formulation.

17. The method of claim 16 wherein steps (b) and (c) are performed in any sequential order or are carried out simultaneously with step (a), and wherein step (d) is carried out last.

18. A method of reducing blood pressure in an obese mammal, the method comprising orally administering to a subject in need thereof a therapeutically effective amount of adipocyte-derived, hydrolyzed proteins and fragments thereof within a magnesium carrier....
### A. CLASSIFICATION OF SUBJECT MATTER

**IPC(8)**: A61K 35/32, 35/36 (201 1.01)

**USPC**: 424/574

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)

USPC: 424/574

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

USPC: 424/130.1: 435/174, 183; 514/12, 17.2 (see search terms below)

Electronic database consulted during the international search (name of data base and, where practicable, search terms used)

PubWEST (PGPB, USPT, EPAB, JPAB)

adipocyte-derived proteins, fat, whole blood, whole milk or egg, embedded, metal salt carrier, magnesium or calcium salt, hydrolyzed, dry-heated, atherosclerosis, obesity, tablet or pill, oral vaccine

### 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>
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<td>Y</td>
<td>US 2002/0044968 A1 (LENGERICH) 18 April 2002 (18.04.2002) abstract; para [001 2]; [001 3]; [0016]; [002η]; [0035]-[0039]; [004η]; [0061]</td>
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<td>Y</td>
<td>US 2004/0048249 A1 (TANG et al.) 11 March 2004 (11.03.2004) table 2, pg 58; para [0061]; [0122]; [0128]; [0133]; [0138]; [0178]; [0196]; [0238]; [0262]; [0272]; [0283]; [0293]; [0300]; [0308]</td>
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<td>Y</td>
<td>DIBIASE et al. Gut Microbiota and Its Possible Relationship With Obesity. Mayo Clin Proc; April 2008, Vol 83(4):460-469; pg 460, col 1, para 2; pg 464, col 1, para 2; pg 465, col 2, para 2 to pg 466, col 1, para 1</td>
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<td>Y</td>
<td>US 2008/0096900 A1 (KAYSER et al.) 24 April 2008 (24.04.2008) para [0013], [0183], [0186], [0188], [0190] [0246], [0253]</td>
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<td>Y</td>
<td>US 4.172,072 A (ASHMEAD) 23 October 1979 (23.10.1979) col 2, ln 54-62; col 3, ln 38-40; col 8, ln 5-25; col 9, ln 1-10</td>
<td>16-17</td>
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<td>Y</td>
<td>TABATA et al. Angiopoietin-like Protein 2 Promotes Chronic Adipose Tissue Inflammation and Obesity-Related Systemic Insulin Resistance. Cell Metabolism, September 2009, Vol 10, pp 178-188; pg 178, col 1, para 1</td>
<td>1-10, 18</td>
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</table>

Further documents are listed in the continuation of Box C.

**A** Special categories of cited documents:

- **“A”** 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

**“K”** document member of the same patent family

Date of the actual completion of the international search: 16 February 2011 (16.02.2011)

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<td>P.O. Box 1450, Alexandria, Virginia 22313-1450</td>
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Authorized officer: Lee W. Young

Form PCT/ISA/2 (second sheet) (July 2009)