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(54) Title: DIETARY COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING INSULIN RESISTANCE

(57) Abstract: The invention relates to formulations of nutritional supplements, and methods for administering those supplements to alter an insulin level, to treat insulin resistance, and/or to treat obesity in a patient in need thereof. The nutritional supplement includes (a) docosahexaenoic acid (DHA), (b) eicosapentaenoic acid (EPA), and (c) optionally, a specific inhibitor of enzyme delta-5 desaturase (D5D).

DIETARY COMPOSITIONS AND METHODS OF USE THEREOF FOR TREATING INSULIN RESISTANCE

FIELD OF INVENTION

The invention relates to nutritional supplements, dietary regimens and related methods of treating conditions associated with insulin resistance.

BACKGROUND OF THE INVENTION

One of the leading causes for increased and excessive accumulation of body fat (obesity) is insulin resistance. In this condition, a patient's muscle cells become less responsive to insulin. As a consequence, the pancreas secretes more insulin than would otherwise be needed to reduce the elevated blood glucose levels as compared to an individual who is not suffering from insulin resistance. As a result of insulin resistance, the average level of insulin in the blood stream increases. This condition is termed hyperinsulinemia. With hyperinsulinemia, a clustering of a number of conditions occurs. These include obesity, type 2 diabetes, cardiovascular disease, and hypertension. This is why the hyperinsulinemia caused by insulin resistance is also termed metabolic syndrome.

There are a number of ways of diagnosing insulin resistance, however, fasting insulin levels has been shown to be an excellent indicator. The level of insulin indicative of insulin resistance is usually considered to be greater than about 15 uIU/ml. Another marker is elevated triglycerides (TG) being greater than 150 mg/dL. Thus an elevated TG level can be considered to be a surrogate marker of insulin resistance.

Insulin resistance leads to obesity as increases in serum insulin also inhibit the activity of hormone-sensitive lipases in adipose cells, thus decreasing the release of stored body fat for energy generation. The same hyperinsulinemia can cause the accumulation of fat in the muscle cells. This increased level of intracellular TG levels in the muscle cells further impairs normal insulin action thus increasing still more the levels of insulin resistance and hyperinsulinemia. Eventually, as a consequence of continued chronic insulin resistance, the insulin-producing cells in

the pancreas begin to burn out after having to continually produce larger than normal amounts of insulin. When that happens, the pancreas cannot secrete sufficient insulin to reduce blood glucose, leading to the development of elevated levels of blood glucose and the onset of type 2 diabetes. The development of insulin resistance often precedes the development of type 2 diabetes by several years.

Insulin resistance is also strongly correlated with an increased incidence of cardiovascular disease and hypertension that is a leading cause of stroke. Thus there is a compelling need to reduce insulin resistance using a dietary supplement that can be used on a lifetime basis.

SUMMARY OF THE INVENTION

The present invention provides nutritional compositions and related analytic methods to determine dosage to reduce insulin resistance in a patient, and, ultimately, treat related diseases and conditions, such as obesity, type 2 diabetes, and cardiovascular disease. At a minimum, the invention provides for reducing the risk of developing those diseases or conditions, and ideally it can be used as a treatment for existing conditions.

Although the exact molecular cause of insulin resistance remains unknown, the inventor believes that it lies in a pre-inflammatory state of the cells of in the adipose tissue. This pre-inflammatory state can be characterized by excess levels of arachidonic acid (AA) that is the molecular precursor to the production of inflammatory eicosanoids. Such inflammatory eicosanoids in the adipose tissue can cause the infiltration of macrophages into the adipose tissue with the subsequent release of inflammatory cytokines such as tumor necrosis factor (TNF) and interleukin-6 (IL-6). TNF, in particular, is associated with decreased insulin signaling that leads to insulin resistance. In addition, it is known that increased TG levels in the muscle cells also interfere with normal insulin signaling. Therefore the molecular treatment for insulin resistance requires the simultaneous reduction of AA in the adipose tissue as well as a reduction of TG levels in the muscle cells.

Pro-inflammatory eicosanoids are derived from the long-chain omega-6 fatty acid known as arachidonic acid (AA). There is no known drug that can reduce AA reduce levels, however there are several natural inhibitors of the enzyme delta-5-

desaturase (D5D) that produces AA. One such molecule is eicosapentaenoic acid (EPA) that is a feedback inhibitor of D5D. Another approach is the use of sesamine lignans and curcumin, which are also known to inhibit D5D activity, as well as chemical or synthetic compounds such as sesamol.

Likewise TG levels can be reduced by increased oxidation of fatty acids. At the molecular level, this requires the activation of a class of intracellular receptors known perioxosmal proliferator activity receptors (PPAR), especially PPAR alpha (PPARa). Once activated, PPARa causes the genetic expression of enzymes in both liver and muscle cells that increase the oxidation of fatty acids. The long-chain omega-3 fatty acid docosahexenoic acid (DHA) is one such natural compound that activates the PPARa. Thus, as disclosed herein, a nutritional composition consisting of EPA (as an inhibitor of AA formation) and DHA as an activator of PPARa is useful as a dual treatment in simultaneously treating two of the biochemical events (increased AA levels in the adipose tissue and increased TG levels in the muscle cells) that lead to the development of insulin resistance. Heretofore undiscovered is the effective ratio of EPA and DHA that can accomplish this dual function and the levels of an appropriate nutritional supplement containing the ratio of these two natural fatty acids that can alleviate insulin resistance. This is the discovery which provides the basis of the inventions disclosed herein.

The ratios and amounts of EPA and DHA required to reduce the biochemical causes of insulin resistance (increased inflammation in the fat cells and increased TG levels in the muscle cells) can be determined by the ratio of AA to EPA in the isolated plasma phospholipids and the extent of the reduction of TG in the serum plasma. The phospholipids of the plasma are found primarily in the low-density and high-density serum lipoproteins. These lipoprotein particles are small enough to leave the plasma compartment and circulate in the interstitial space between the endothelial cells that line the vascular system and the lymphatic system where they can be taken up by muscle cells and adipose cells. Thus the AA/EPA ratio in the isolated plasma phospholipids is an indicia of the levels of the same fatty acids in the target tissues of the cells directly affected by insulin resistance. Methods according to the invention include administering adequate levels of the invention to lower the AA/EPA ratio in the plasma phospholipids below about 6. It has been

found that above this level, even with a calorie restricted, low-glycemic diet and exercise (the two primary lifestyle factors used in the treatment of insulin resistance), that adequate benefits of these usual lifestyle recommendations to the obese or overweight patients the reduction of insulin resistance will not be achieved.

In some embodiments, the invention comprises, or consists essentially of, as active ingredients DHA and EPA. Each of those ingredient are in an amount and/or ratio in the supplement such that, in an adult human, the supplement activates the Peroxisome Proliferator-Activated Receptor alpha (PPARa) and reduces the amount of pro-inflammatory eicosanoids. Other embodiments can include other inhibitors of D5D such as sesamine lignans or curcumcin, or the addition of a synthetic compound such as, for example, sesamol. The levels required to reduce insulin resistance can be readily determined from the combined ratios of AA/EPA and TG/HDL

In one embodiment, methods according to the invention include administering orally to the patient at least about one gram of DHA and at least about one 1.5 grams of EPA, preferably over a 24-hour period. Alternatively, administration over shorter or longer periods can be effective and are contemplated by the methods of the present invention.

Methods according to the invention also include establishing and/or monitoring at least two clinical markers: the ratio of arachidonic acid to eicosapentaenoic acid (AA/EPA) and the levels of TG. The AA/EPA should be less than about 6 and the levels of TG should be less than about 130 mg/dL as indicia of a reduction in insulin resistance.

Methods according to the invention can further include adjusting the nutritional composition based on at least one of the above clinical markers. Preferably, the relative and absolute amounts of each of DHA and EPA present in the nutritional composition are effective for optimizing the reduction of insulin resistance in a patient by simultaneously reducing the AA/EPA ratio in the adipose tissue and activating the PPARa using DHA as its agonist, thus reducing the levels of TG in the muscle cells.

Methods according to the invention can be used to reduce insulin resistance in a patient and/or can be used to treat, without limitation, obesity, type 2 diabetes, and cardiovascular disorders including hypertension. The invention further features methods of preparing the nutritional composition according to the invention, including the steps of providing a source of DHA, providing a source of EPA, and optionally providing an additional source of a specific D5D-inhibitor such as a lignan from sesame oil, curcumin or a synthetic compound(s) known to inhibit D5D. One preferred synthetic compound is sesamol. The nutritional composition according to the invention can be administered to patients for long-term prevention of the development of insulin resistance since EPA and DHA are essential fatty acids necessary for human health. The nutritional composition can also be administered as a medicinal treatment to human patients requiring treatment for conditions associated with insulin resistance. These would include obesity, type 2 diabetes, cardiovascular disease, hypertension, polycystic ovary syndrome, neurological disorders and cancer. The nutritional supplement may also be administered to non-human mammals for veterinary or dietary purposes. According to one aspect of the present invention, a method for decreasing the level of insulin resistance in a patient comprises administering, to a patient in need of treatment of insulin resistance, a nutritional supplement comprising (a) a minimum of about 1 gram daily of docosahexaenoic acid (DHA), and (b) a minimum of about 1 gram of eicosapentaenoic acid (EPA). It is contemplated herein that a patient is administered at least one gram each of EPA and DHA in total over a 24 hour period. Shorter or longer periods of administration can also be effective. In certain preferred embodiments, the DHA and EPA are provided in an oil extracted from a fish.

In one currently preferred embodiment, the total concentration of mercury in said nutritional supplement is less than about 10 ppb. In another embodiment, the total concentration of PCBs in said nutritional supplement is less than about30 ppb. In another embodiment, the total concentration of dioxins in said nutritional supplement is less than about 1 ppt.

Other currently preferred embodiments of the present invention relate to a nutritional supplement which further comprises an inhibitor of D5D that is not EPA. Preferably, such an inhibitor can be extracted from a natural source or alternatively is in a concentrated, purified form. Thus, in accordance with the present invention, a method for decreasing the level of insulin resistance in a patient comprises administering, to a patient in need of treatment of insulin resistance, a nutritional supplement comprising (a) a minimum of about 1 gram daily of docosahexaenoic acid (DHA), and (b) a minimum of about 1 gram of eicosapentaenoic acid (EPA); and optionally, an additional inhibitor of D5D enzyme activity. In certain embodiments, the optionally added inhibitor of D5D is extracted from sesame oil. In other embodiments, the optionally added in inhibitor of D5D comprises a lignan. In yet other preferred embodiments, the optionally added inhibitor of D5D is selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol, and sesamolin. In still others, the optionally added inhibitor of D5D is curcurmin. Alternatively, the optionally added inhibitor can be a synthetic compound, such as but not limited to, sesamol.

According to the present invention, the effective amount of EPA and/or EPA plus an additional D5D inhibitor is sufficient to reduce the ratio of AA/EPA in isolated phospholipids to less than about 6.

According to the present invention, it is contemplated that the nutritional supplement is administered enterally or parenterally. Moreover, it is contemplated that the nutritional supplement is in a form selected from the group consisting of a capsule, an edible solid, a tablet, a powder, and a beverage.

In certain preferred embodiments, the nutritional supplement is an edible solid. An edible solid can be preferably in the form of bar, but other forms of edible solids are contemplated herein.

In another aspect, the present invention relates to a nutritional supplement comprising (a) a minimum of about 1 gram daily of docosahexaenoic acid (DHA), and (b) a minimum of about 1 gram of eicosapentaenoic acid (EPA); and, optionally, an additional inhibitor of D5D enzyme activity . The particulars for each of the foregoing active ingredients are set forth elsewhere herein.

BRIEF DESCRIPTION OF THE DRAWING

FIG. 1 illustrates metabolic pathways for conversion of omega 6 and omega 3 essential fatty acids into eicosanoids.

DETAILED DESCRIPTION OF THE INVENTION

Although the cause(s) of insulin resistance in muscle cells have remained heretofore unknown, the teachings set forth herein provide evidence of a discovery that points to increased accumulation of pro-inflammatory eicosanoids derived from AA in the adipose tissue coupled with decreased activity of gene-regulating elements, such as PPARa. In particular, PPARa acts as a ligand-activated transcription factor that regulates the expression of genes that improve oxidation of fatty acids thus reducing the levels of triglycerides. Therefore, the present invention teaches the primary approach of devising treatments that simultaneously activate PPARa and reduce the production of pro-inflammatory eicosanoids by reducing the ratio of AA/EPA in the adipose tissue. The ratio of AA/EPA in the plasma phospholipids is a surrogate marker for the ratio of AA/EPA in the adipose tissue.

PPARa is found primarily in the liver and muscles. Drugs known to act as agonists of PPARa include fibrates. The present invention provides at least one natural agonist of PPARa as part of a nutritional supplement. However, certain preferred embodiments of the present invention use an agonist extracted or purified from natural sources, to activate the PPARa. In particular, one preferred nutritional supplement formulations according to the invention utilizes one or more of the essential fatty acids that can be found in a natural diet as part of the supplement. The long-chain omega-3 fatty acid, DHA, has been found to activate PPARa. At adequate levels, dietary DHA is able to act as an agonist of PPARa and therefore help reduce insulin resistance by activating the oxidation of TG in target cells such as the muscle. Because activation by omega-3 fatty acids including DHA on PPARa appears to be relatively weak, in some other preferred embodiments, a high dose of DHA, e.g., at least about 1 gram per day, is formulated as part of the nutritional supplement according to the invention.

Since insulin resistance is multi-factorial, other potential causes and factors are taken into consideration to devise an effective remedy as disclosed herein. Accordingly, another long-chain omega-3 fatty acid, EPA, is also included as part of one preferred nutritional supplement according to the invention because it has been shown that EPA can reduce the production of AA by inhibiting the enzyme delta 5 saturase (D5D). As stated above, pro-inflammatory eicosanoids derived from AA appear to play a role in insulin resistance because of their ability to recruit macrophages with the subsequent release of pro-inflammatory cytokines such as TNF and IL-6 which are known to be associated with development of insulin resistance. Therefore, the supplement according to the invention also treats insulin resistance by reducing inflammation in the adipose tissue through increased EPA intake.

Fish oil is rich in both long-chain omega-3 fatty acids, DHA and EPA, and is included in certain preferred embodiments of the invention in order to activate PPARa as transcription factors, and to reduce the amount of inflammatory cytokines such as TNF in the patient body. Since high doses of fish oil are required, it is necessary to purify to exceptionally standards that reduce the likelihood of build-up of toxins such as PCBs and dioxins that are present in all fish oils. In a preferred embodiment, a patient suffering from insulin resistance and/or suffering from a disease associated with insulin resistance such as obesity or diabetes, is administered at least about 1 gram of DHA and at least about 1 gram of EPA, optionally at least about 1 to 4 grams of EPA, over a 24-hour period on a continual basis. Because of the need to take high doses of DHA and EPA, the nutritional supplement of the present invention is formulated to be low in toxins like mercury (less than about 10 ppb), PCBs (less than about30 ppb), and dioxins (less than about 1 ppt).

The term "nutritional supplement," as used herein, is intended to encompass administration of the supplement by enteral or parenteral methods, as well as oral administration, and includes without limitation, vitamin supplements, dietary supplements, and/or medicinal supplements, in liquid forms, solid forms or a combination thereof. Nutritional supplements as contemplated herein can take a variety of forms, e.g., a capsule, an edible solid such as but not limited to a bar, a tablet, a powder, a beverage for example but not limited to a beverage package, or

an injectable formulation, as would be known to and judged suitable by a practitioner skilled in the art. By way of example, nutritional supplements according to the invention are easily administered to a patient by enteral administration, preferably by oral administration, as a food product. The food product can be a solid, a liquid, or a combination thereof. Alternatively, nutritional supplements are in a form conventionally known to those skilled in the art as suitable for administering oil-based vitamins and metabolites, such as, without limitation, encapsulation within a soft gelatin capsule. In some embodiments, some or all of the active ingredients of a supplement are to be taken relatively close to each other in time. In certain embodiments of the present invention, each of the active ingredients can be admixed to form a single supplement or can be separate ingredients administered coincidentally in accordance with the teachings set forth herein.

Thus, nutritional supplements according to the invention can be administered to a patient in a manner known to those skilled in the art, including enteral and parenteral administration. Without limitation, nutritional supplements according to the invention are preferably administered by enteral means, and, more preferably, is administered by oral ingestion.

In certain embodiments, a supplement according to the present invention comprises, or alternatively consists essentially of, the following active ingredients: DHA, EPA, and optionally a specific D5D-inhibitor. The supplement can include an oil containing both EPA and DHA, and the oil may be extracted from part of a fish, e.g., cod liver or some small marine animals such as krill. The EPA and DHA can also be isolated from algae sources. The specific D5D-inhibitor can be lignans extracted from sesame oil or other natural compounds such curcumin. The specific D5D inhibitor can also be a synthetic chemical such sesamol. This combination of EPA and another D5D inhibitors enhances production of AA thus lowering the levels of pro-inflammatory mediators in the adipose tissue.

As taught herein, the amount and ratio of various components of a supplement of the present invention can be sufficient to reduce both the AA/EPA ratio to below about 6 in the plasma and the levels of TG to less than about 130 mg/dL in the plasma. This requires a nutritional supplement of the present invention to contain a minimum of about 1 gram of DHA and between about 1-4 grams of

EPA, preferably a minimum of about 1 gram EPA, as well as low concentrations of potential toxins such as mercury, PCBs, and dioxins as described elsewhere herein. The effective amounts of EPA can be reduced further by adding an appropriate D5D inhibitor. The final percentage of the additional D5D inhibitor can be up to about 15% by weight of the nutritional supplement.

The AA/EPA ratio in the plasma phospholipids is a reliable marker of the levels of the same fatty acids in the adipose tissue. AA is the essential fatty acid precursor to virtually all pro-inflammatory eicosanoids, which, in turn, induces the production of pro-inflammatory cytokines implicated in insulin resistance, whereas eicosanoids derived from EPA have little, if any, pro-inflammatory function.

Accordingly, the formulation of a nutritional supplement in accordance with the present invention can be varied or adjusted, e.g., customized, preferably optimized for effectiveness, to the physiological needs or requirements of a particular patient (or particular group of patients exhibiting similar characteristics) to whom the nutritional supplement is intended to be administered. Preferably, the relative and absolute amounts of each of the active ingredients in the nutritional supplement according to the invention are effective in reducing insulin resistance in a patient by activating the gene-regulating functions of PPARa using DHA but without the stimulation of pro-inflammatory eicosanoids and cytokines. All that is required is a reduction in the level of insulin resistance and the skilled practitioner will recognize how to vary the actual formulation of the nutritional supplement described herein to accomplish such a reduction.

For example, in certain embodiments, amounts and/or ratios of each of the active ingredients, i.e., EPA and DHA, and optionally additional specific D5D-inhibitors, are established in the nutritional supplement according to the invention so as to maintain the AA/EPA ratio in the plasma phospholipids about 6 or below to be consistent with the necessary reduction of inflammation in the adipose tissue to reduce the severity of insulin resistance. In addition, the levels of DHA must be high enough to activate PPARa in order to induce the increased oxidation of triglycerides in muscle cells. The indication of the appropriate level is the reduction of serum TG levels to about 130 mg/dL or less. The invention features methods that seek to prevent the development of insulin resistance or to treat a patient who has a

medical condition associated with insulin resistance. For example, the patient may have been diagnosed as suffering from insulin resistance, e.g., through a clinical test known to a physician skilled in the art. For example, the patient may have greater than about a 15 uU/ml fasting insulin level. Alternatively, the patient may be suffering from one or more conditions or diseases associated with insulin resistance, such as obesity, type 2 diabetes or other conditions associated with insulin resistance and/or metabolic syndrome.

Methods according to the invention include administering to the patient a nutritional supplement according to the invention described herein. The supplement, in some embodiments, includes DHA, EPA, and optionally another inhibitor of D5D, thereby lowering the severity of insulin resistance in the patient. In a preferred embodiment, the nutritional supplement and method of use thereof restores insulin sensitivity and can thereby effectively also ameliorate conditions associated with insulin resistance in the patient.

Optionally, methods according to the invention also include monitoring one or more patient health indicators and/or adjusting the formula of the nutritional supplement. For example, an increase in the AA/EPA ratio would indicate elevated inflammation in the adipose tissue. This would indicate the need for a higher amount of EPA relative to DHA for that patient. Elevated levels of TG indicate a greater amount of the invention (and hence greater intake of DHA) to activate the PPARa receptors is required to increase fatty acid oxidation in the muscle and liver.

Preferably, monitoring the AA/EPA and levels of TG includes obtaining a sample of blood plasma phospholipids from the patient, and measuring the amount of the fatty acids and lipids implicated in the ratio as described in detail below in the "Methods and Examples" section.

Thus, the instant invention provides methods for reducing insulin resistance in a patient. Methods and supplements according to the invention can be used to treat, without limitation, obesity, type 2 diabetes and cardiovascular disorders, metabolic syndrome, and other conditions that may be associated with insulin resistance.

In another aspect, the delivery of the invention can be in the form of a food product that includes a nutritional supplement according to the invention. Preferably, the food product contains between about 1 and about 60 grams of carbohydrate and between about 1 and about 40 grams of protein. More preferably, protein and carbohydrate are present in the food product at a ratio of between about 0.5 and about 1.0 of protein to carbohydrate, inclusive. This will lower the insulin secretion and thus reduce the activating impact that insulin has D5D activity. Food products of the invention can include, for example, a food bar, a confection product, ice cream, a beverage, e.g., a ready to drink mix, a convenience food, e.g., a frozen meal, or a stabilized meal. The foregoing are exemplary and non-limiting embodiments. The skilled practitioner will recognize the utility of other embodiments.

Nutritional supplements according to the invention can be administered to patients for long-term healthy dietary maintenance, and/or nutritional supplements can be administered as a medicinal treatment to human patients requiring treatment for conditions associated with insulin resistance. The methods and nutritional supplements described here in may be administered to humans and to non-human mammals for veterinary or dietary purposes or for the purpose of evaluating suitability for human administration.

METHODS AND EXAMPLES

Preparation of a Nutritional Supplement:

EPA and DHA can each be in the form of a triglyceride. Other acceptable forms include but are not limited to methyl or ethyl esters, long-chain wax esters, monoglycerides, phospholipids, free fatty acids, or the appropriate salts of free fatty acids.

EPA and DHA: Fish oil containing both EPA and DHA can be easily extracted from natural sources such as plankton, krill, fish or other marine animals. Such isolated fish oils can be blended to provide the appropriate balance of DHA and EPA required for the invention. EPA and DHA also can be fermented from algae under controlled conditions. In both cases, the extracted oil can be refined to meet all international standards for edible oils as well as the more rigid requirements

of decreased levels of mercury, PCBs and dioxins. The triglyceride form of fish oil can be altered either by chemical or biochemical means to produce free fatty acids, salts of free fatty acids, methyl or ethyl esters, long-chain wax esters, phospholipids, or monoglycerides which can be further fractionated to give higher contents of EPA and DHA than the starting oil in the triglyceride form. EPA and DHA can also be chemically synthesized.

The invention requires a minimum of about1 gram per day DHA to activate the PPARa receptor and at least about one gram of EPA to reduce the activity of the D5D enzyme. A preferred dosage is about 1 gram of DHA and about 2 grams of EPA per day. Another preferred dosage is about 1 gram of DHA and about 1-4 grams of EPA per day.

The measurement of the AA/EPA ratio to determine the levels of EPA from the isolated phospholipids of the lipoprotein fractions is preferred over other methodologies. First, the patient does not have to be in a fasted state. Second, the fatty acids in the plasma lipoprotein are in dynamic interaction with the lymphatic circulation, whereas red blood cell membranes are not. Finally, the absence of hemoglobin in the plasma lipoprotein fractions reduces the likelihood of auto-oxidation of the highly polyunsaturated long-chain omega-3 fatty acids, thus avoiding erroneous results on the actual levels of inflammation and insulin resistance in the patient.

Specific D5D-inhibitor: Specific D5D-inhibitors useful in the invention to reduce the levels of EPA required to include, for example, an extract of sesame oil that is rich in one or more lignans such as, without limitation, sesamin, episesamin, sesaminol, episesaminol, and sesamolin. It can also include curcurmin, and a synthetic chemical such as but not limited to sesamol. In certain embodiments, a preferred synthetic ingredient is sesamol.

Forms of sesame oil useful as a source of specific D5D-inhibitor include crude or refined sesame, prepared by methods known to those skilled in the art of nutritional supplements. Also useful in the invention are distillates of sesame oil, extracts, and lignan preparations crystallized from acetone.

Steam distillation of refined sesame oil provides a distillate enriched in lignans. Sesame oil distillates can be prepared by subjecting crude or refined sesame oil to steam sparging at high vacuum conditions (<0.5 mmHg) and 300-350°F to strip off the unsaponifiable fraction as a distillate. The distillate is then recovered in a condenser placed immediately after the stripping operation.

Unsaponifiables rich in lignans can be recovered from sesame oil distillate. Acetone fractionation of the sesame oil distillate can further enrich the lignans. The final product can be concentrated by crystallization from acetone. Alternatively, it can be isolated by super critical fluid technology. To achieve a higher concentration of the lignan, enzymatic hydrolysis of the remaining triglycerides in the distillate fraction can be used to convert the triglycerides to free fatty acids and glycerol. This process is then followed by molecular distillation to remove the glycerol and free fatty acids in order to leave a fraction much richer in lignans. The unsaponifiable fraction of sesame oil will be rich in lignan compounds including, without limitation, sesamin, sesamolin, sesaminol, and episesamin. To this fraction can be added other D5D inhibitors such as sesamol.

The invention containing DHA (for activating the PPARa receptor) and EPA (to inhibit the D5D enzyme) can lower the AA/EPA in the blood plasma phospholipids to about 6 or less and the fasting triglycerides to less than about 130 mg/dL to be considered effective.

Method of Monitoring Patient

Clinical monitoring of blood plasma phospholipids: Levels of essential fatty acids were measured in blood plasma phospholipids by drawing a blood sample from a patient subject. The plasma was separated by centrifugation from the cellular components of the blood sample. The plasma was then extracted with a 2:1 ratio of chloroform to methanol, and the lower phase was taken to dryness under nitrogen, by methods known to those skilled in the art.

Lipid was extracted from the plasma with a known amount of diheptadecanoyl phosphatidylcholine (17:0 PC). The total lipids were extracted using a 2:1 mixture of chloroform/methanol mixture of which four parts was added to one part of plasma. After separation, the lower phase was retained and taken to dryness. The dried lipids were separated on a silica gel thin layer plate to separate

the phospholipids from the other lipid components in the extracted serum, by spotting the concentrated lipid extract on standard silica gel G plates. Thin-layer chromatographic development in heptane-isopropyl ether-acetic acid (60:40:3) was used to isolate the phospholipid portion. The phospholipids were removed, and the component fatty acids were methylated with boron trichloride in methanol.

Following extraction, the phospholipids were analyzed by a gas chromatograph to determine the lipid profile of the phospholipids. From that lipid profile, the ratio of AA/EPA is determined. Capillary gas-liquid chromatography of the fatty acid methyl esters was performed using a Varian 3800 gas chromatograph (Palo Alto, California) with a 60 m DB-23 capillary column (0.32 mm internal diameter), to determine the lipid profile of the phospholipids. The integrated areas under the curve of those peaks known to correspond to AA and EPA were calculated by standard procedures known to those skilled in the art. The ratio of the integrated area of a fatty acid peak to the integrated area of another fatty acid peak was then calculated to determine the aforementioned required ratios in the isolated plasma phospholipids.

The measurement of the triglycerides was done under fasting conditions using analytical techniques well known to one skilled in the art.

Use of the Nutritional Supplement in a Food Product:

As seen from FIG. 1, the hormone insulin can also activate the enzyme D5D. Therefore, if a nutritional supplement according to the invention is to be incorporated into a food format, then the ratio of protein to carbohydrate can be adjusted to enhance its efficacy. This is because, at a low protein-to-carbohydrate ratio, excess insulin can be generated, and the increased insulin can stimulate the D5D activity to produce more AA, thereby increasing the level of insulin resistance though increases in pro-inflammatory cytokines and eicosanoids. The preferred ratio of protein-to-carbohydrate for a food product that incorporates the nutritional composition of this invention is between about 0.5 and about 1.0, inclusive, and the carbohydrate content should preferably have a low glycemic index for maximum insulin control.

The composition of this invention can be included in a wide variety of formats including but not limited to liquids, soft gelatin capsules, dry microcapsules, food bars, ready to drink mixes, ice creams, margarines, and other food formats into which oils or dry microencapsulated oils can be easily incorporated.

EXAMPLE 1

An example of the invention to treat insulin resistance can be found in pediatric obesity. 43 children (ages 10-18) with pediatric obesity (greater than 95th percentile of height-weight for their age) were randomized into two groups. A BMI-Z score represents greater than two standard derivations from the normal BMI for their age group. A number greater than 2 indicates greater than the 95th percentile of height-weight for their age. Both groups received the same dietary advice on following a low glycemic load diet and were provided additional exercise training. One group was provided 6 grams of the invention in six soft gelatin capsules providing a total of 1.2 grams of DHA and 2.4 grams of EPA on a daily basis. The baseline characteristics of the two groups are shown in Table 1.

Table 1. Baseline characteristics of pediatric children.

Parameter	Values
BMI	34.61±6.1
BMI-Z	2.39±0.3
Insulin (uU/ml)	22.87±11.7
Triglycerides	162.83±92.9
AA/EPA	25.41±12.8

The data in Table 1 indicate that the children had insulin resistance (insulin levels greater than 15 uU/ml) had elevated levels of triglycerides (greater than 150 mg/dL) and an elevated level of inflammation (AA/EPA greater than 12). The marker of BMI-Z was used, as it is an indication of the standard deviation from normal height-weight charts to take into account the growth in height of the children relative to

their weight. A BMI-Z score of greater than 2 indicates that the subject was more than two standard derivations for a normal height-weight of a child of that age.

The baseline characteristics indicated that the children were obese (as defined by a BMI-Z greater than two), they had insulin resistance (as indicated by elevated insulin levels), and were demonstrating metabolic syndrome (as indicated by their elevated levels of triglycerides), and had significant levels of inflammation (as indicated by the elevated AA/EPA levels).

The children were randomized into two groups and given the same dietary and exercise instruction. The only difference was the level of the invention provided to them as a supplement. The results at six weeks are shown in Table 2.

Table 2.

BMI-Z score	Week 0	Week 6	Statistical significance
Diet + invention	2.31±0.4	2.29±0.4	p = 0.034
Diet only	2.42±0.3	2.40±0.3	n.s.
Insulin			
Diet + invention	21.76±12.9	20.35±9.2	p=0.003
Diet only	26.34±10.3	31.04±15.2	n.s.
Triglycerides			
Diet + invention	159.82±64.5	129.5±48.5	p=0.038
Diet only	173.06±122.8	183.7±107.9	n.s.
AA/EPA			
Diet + invention	28.88±14.9	4.70±2.7	p < 0.0001
Diet only	25.05±9.9	35.45±17.1	n.s.

n.s. = not significant

The data indicate that high levels of DHA (to activate the PPARa receptor to induce increased oxidation of fatty acids) and EPA (to lower the levels of inflammation) were required to statistically reduce the insulin resistance (as indicated by the fasting insulin levels and triglycerides) in children with pediatric obesity.

EXAMPLE 2

Five overweight individuals were placed on a calorie restricted, low glycemic load diet and provided with the invention as a nutrition supplement. The invention consisted of the daily intake of 8 one-gram capsules containing 1.6 grams of DHA and 3.2 grams of EPA. The results after four weeks are shown in Table 3.

Table 3.

Parameter	Start	4 Weeks	Significance
Weight	255.8	247.9	n.s.
Triglycerides	211.8	139.4	p=0.017
AA/EPA	24.94	7.85	p=0.006

n.s.= not significant

The subjects were clearly obese at the start of the experiment. After four weeks of a highly controlled low glycemic load diet, there was no significant change in their body weight. Likewise the level of DHA in the invention was not sufficient to reduce the triglyceride levels to the desired levels (about 130 mg/dL or less) or the level of inflammation as measured by the AA/EPA (about 6 or less) although the changes were statistically significant. This indicates that the amount of the DHA and EPA in this embodiment of the invention for these overweight adults can be increased to a higher level to reduce insulin resistance. This confirms the importance of monitoring such clinical parameters to determine the effective levels of the invention to reduce insulin resistance.

EXAMPLE 3

An exemplary method of preparing a D5D inhibitor is as follows. Forty (40) kg of refined sesame oil was distilled to provide 800 ml of distillate containing lignans from sesame oil. And 20 ml of distillate was added to 100 ml of acetone and cooled to –8 °C. The solution was filtered to provide 5 ml of fractionated distillate rich in lignans.

EXAMPLE 4

An exemplary method of preparing a nutritional supplement with all three active ingredients is as follows. Such a nutritional supplement was prepared by combining 100 ml of a fish oil concentrate (containing about 40% of the total fatty acids as EPA and about 20% as DHA) with about 1% by weight of the sesame lignan extraction in Example 3.

EXAMPLE 5

The composition in Example 4 was, optionally, encapsulated in a soft gelatin capsule suitable for enteral administration.

EXAMPLE 6

An exemplary method of preparing a food product is as follows. The composition in Example 4 was micro-encapsulated as a dry powder and incorporated into a food bar containing about 14 grams of protein and about 20 grams of low-glycemic index carbohydrate and about 7 grams of fat. Alternatively, the supplement according to the instant invention can be added to one or more of the food products disclosed in U.S. Publ. Appln. No. US 2001/0022980 Al, published September 20, 2001, and in U.S. Patent No. 5,902,797, issued May 11, 1999, the entire content of each of which is incorporated herein by reference.

EXAMPLE 7

An exemplary method of determining certain clinical indicators useful in formulating and adjusting the nutritional supplement according to the invention is as follows. A blood sample of 100 microliters was drawn from the patient. Plasma was separated from the cellular components of the blood by centrifugation. The plasma was extracted with a 2:1 ratio of chloroform to methanol, and the lower phase was taken to dryness under nitrogen. The dried lipids were separated on a silica gel thin layer plate to separate the phospholipids from the other lipid components of the extracted serum. The phospholipids were then analyzed by a gas chromatograph to determine the lipid profile of the phospholipids. From that lipid profile, the ratio of AA/EPA was determined.

While this invention has been particularly shown and described with reference to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention as defined by the claims. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described specifically herein. Such equivalents are intended to be encompassed in the scope of the claims.

What is claimed is:

1. A method for decreasing the level of insulin resistance in a patient, the method comprising the step of:

administering, to a patient in need of treatment of insulin resistance, a nutritional supplement comprising (a) a minimum of about 1 gram daily of docosahexaenoic acid (DHA), and (b) a minimum of about 1 gram of eicosapentaenoic acid (EPA).

2. The method of claim 1 in which the total concentration of mercury in said nutritional supplement is less than about 10 ppb.

3. The method of claim 1 in which the total concentration of PCBs in said nutritional supplement is less than about 30 ppb.

4. The method of claim 1 in which the total concentration of dioxins in said nutritional supplement is less than about 1 ppt.

5. The method of claim 1 wherein the nutritional supplement further comprises an inhibitor of D5D that is not EPA wherein said inhibitor can be extracted from a natural source or is in a concentrated, purified form.

6. The method of claim 1 wherein the DHA and EPA are provided in an oil extracted from a fish.

7. The method of claim 1 comprising administering to the patient at least one gram each of EPA and DHA in total over a 24 hour period.

8. A method for decreasing the level of insulin resistance in a patient, the method comprising the step of:

administering, to a patient in need of treatment of insulin resistance, a nutritional supplement comprising (a) a minimum of 1 gram daily of docosahexaenoic acid (DHA), and (b) a minimum of 1 gram of eicosapentaenoic acid (EPA); and optionally, an additional inhibitor of D5D enzyme activity

9. The method of claim 8 wherein the inhibitor of D5D is extracted from sesame oil.

10. The method of claim 8 wherein the inhibitor of D5D comprises a lignan.

11. The method of claim 8 wherein the inhibitor of D5D is selected from the group consisting of sesamin, episesamin, sesaminol, episesaminol, and sesamolin.

12. The method of claim 8 wherein the inhibitor of D5D is curcurmin.

13. The method of claim 8 wherein the inhibitor of D5D is a synthetic compound such as sesamol.

14. The method of claim 1 or 8 wherein the effective amount of EPA and/or EPA plus an additional D5D inhibitor is sufficient to reduce the ratio of AA/EPA in isolated phospholipids to less than about 6.

15. The method of claim 1 or 8 wherein the nutritional supplement is administered enterally.

16. The method of claim 1 or 8 wherein the nutritional supplement is administered parenterally.

17. The method of claim 1 or 8 wherein the nutritional supplement is in a form selected from the group consisting of a capsule, an edible solid, a tablet, a powder, and a beverage.

18. The method of claim 1 or 8 wherein the nutritional supplement is an edible solid.

19. The method of claim 1 or 8 wherein the edible solid is in a bar form.

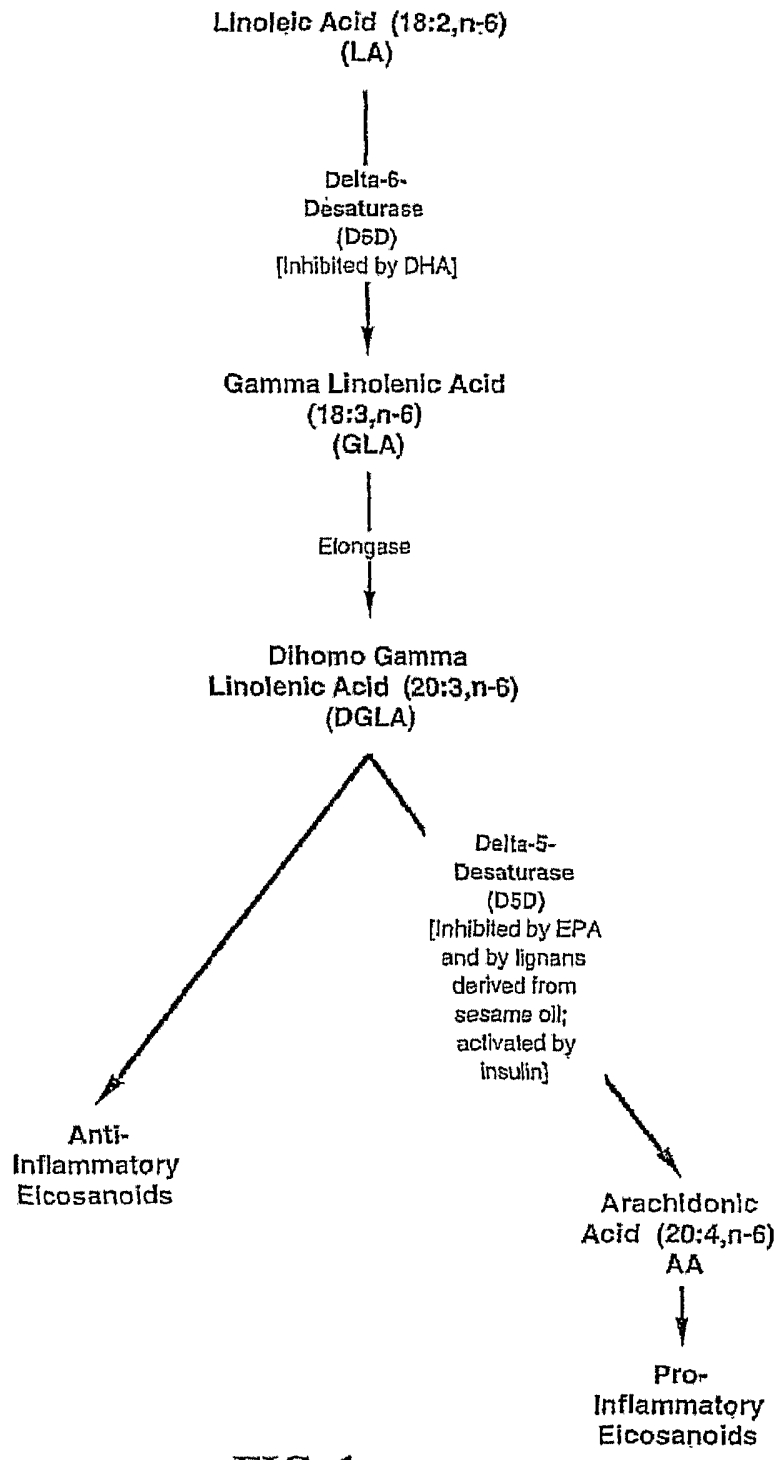
Linoleic Acid (18:2,n-6)
(LA)
Delta-6-
Desaturase
(D6D)
[Inhibited by DHA]
Gamma Linolenic Acid
(18:3,n-6)
(GLA)
Elongase
Dihomo Gamma
Linolenic Acid (20:3,n-6)
(DGLA)
Delta-5-
Desaturase
(D5D)
[Inhibited by EPA
and by lignans
derived from
sesame oil;
activated by
insulin]
Anti-
Inflammatory
Eicosanoids
Arachidonic
Acid (20:4,n-6)
AA
Pro-
Inflammatory
Eicosanoids

FIG. 1