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Titre : NOUVEAUX MARQUEURS ET CIBLES METABOLIQUES
Title: NOVEL METABOLIC TARGETS AND MARKERS

Abrégé/Abstract:
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NOVEL METABOLIC TARGETS AND MARKERS

RELATED APPLICATIONS

The present application claims priority under 35 U.S.C. §119(e) from provisional application numbers 60/363,587, filed March 11, 2002; 60/373,912, filed April 19, 2002; 60/401,684, filed August 6, 2002; 60/424,949, filed November 8, 2002; and 60/436,192 filed December 24, 2002.

FIELD OF THE INVENTION

This invention relates generally to the field of metabolic targets and markers, especially their therapeutic and diagnostic applications.

BACKGROUND OF THE INVENTION

Metabolites and metabolic pathways are closely associated with biological conditions and functions in a system, e.g., human. Several disease conditions or conditions susceptible to certain diseases can be linked with the levels of certain metabolites or metabolic profiles. In addition, certain metabolites are believed to be the cause of various abnormal conditions. Therefore, there is a need in the field to identify metabolic markers and targets for therapeutic or diagnostic applications.

SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that certain metabolites or metabolic pathways can be used as diagnostic or therapeutic markers. For example, phosphatidylethanolamine-N-methyltransferase (PEMT) activity and other metabolic activities or markers associated therewith can be used either as markers for diagnosing various conditions or as targets for therapeutic treatment of various disease conditions.

In one embodiment, the present invention provides a method for regulating the level of a fatty acid in a system. The method includes regulating the phosphatidylethanolamine-N-methyltransferase (PEMT) activity in the system whereby regulating the level of a fatty acid in the system, wherein the fatty acid is selected from the group consisting of 16:0, 18:0, 16:1n7, 18:1n7, 18:2n6, 20:4n6,
20:5n3, 22:6n3, saturated fatty acids, polyunsaturated fatty acids, and highly unsaturated fatty acids.

In another embodiment, the present invention provides a method for decreasing the level of a fatty acid in a system. The method includes decreasing the CDP-choline activity in the system whereby decreasing the level of a fatty acid in the system, wherein the fatty acid is selected from the group consisting of 16:0, 16:1n7, 18:2n6, and saturated fatty acids.

In yet another embodiment, the present invention provides a method for increasing the PEMT activity in a subject. The method includes administering to the subject an effective amount of an agent selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

In still another embodiment, the present invention provides a method for regulating a lipoprotein component ratio in a system. The method includes regulating the PEMT activity in the system whereby regulating the lipoprotein component ratio in the system, wherein the lipoprotein component ratio is selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine.

In another embodiment, the present invention provides a method of regulating the density or size of a lipoprotein in a system. The method includes regulating a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system whereby regulating the density of a lipoprotein in the system.

In another embodiment, the present invention provides a method for regulating the density or size of a lipoprotein in the system. The method includes regulating the PEMT activity in the system whereby regulating the density of a lipoprotein in the system.

In another embodiment, the present invention provides a method of assessing the density of a lipoprotein in a system. The method includes determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free
cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system wherein the lipoprotein component ratio is inversely related to the density of the lipoprotein in the system.

In another embodiment, the present invention provides a method of assessing the size of a lipoprotein in a system. The method includes determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system wherein the lipoprotein component ratio correlates with the size of the lipoprotein in the system.

In yet another embodiment, the present invention provides a method of assessing the density of a lipoprotein in a system. The method includes determining the PEMT activity in the system, wherein the PEMT activity is inversely related to the density of the lipoprotein in the system.

In another embodiment, the present invention provides a method of assessing the size of a lipoprotein in a system. The method includes determining the PEMT activity in the system, wherein the PEMT activity correlates with the size of the lipoprotein in the system.

In yet another embodiment, the present invention provides a method of assessing the density of a lipoprotein in a system. The method includes determining the homocysteine level of the system, wherein the homocysteine level correlates with the density of the lipoprotein in the system.

In another embodiment, the present invention provides a method of assessing the size of a lipoprotein in a system. The method includes determining the homocysteine level of the system, wherein the homocysteine level is inversely related to the size of the lipoprotein in the system.

In another embodiment, the present invention provides a method for treating or preventing a cardiovascular condition. The method includes administering to a subject in need of such treatment an agent, wherein the agent regulates a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, triacylglyceride to phosphatidylcholine, and S-adenosylmethionine to S-adenosylhomocysteine.
In yet another embodiment, the present invention provides a method for treating or preventing a cardiovascular condition. The method includes administering to a subject in need of such treatment an agent, wherein the agent regulates the PEMT activity.

In still another embodiment, the present invention provides a method for treating or preventing a cardiovascular condition. The method includes administering to a subject in need of such treatment an effective amount of folic acid, estrogen, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

In yet another embodiment, the present invention provides a method for treating or preventing a cardiovascular condition. The method includes administering to a subject in need of such treatment an effective amount of an agent, wherein the agent regulates the level of 22:6n3 or 20:4n6.

In another embodiment, the present invention provides a method for assessing the susceptibility of a subject to a cardiovascular condition. The method includes determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to phosphatidylcholine, triacylglyceride to phosphatidylcholine in the subject, wherein a lipoprotein component ratio that is higher than a normal lipoprotein component ratio is indicative of the susceptibility of the subject to the cardiovascular condition.

In yet another embodiment, the present invention provides a method for assessing the susceptibility of a subject to a cardiovascular condition. The method includes determining the PEMT activity in the subject, wherein a PEMT activity that is lower than a normal PEMT activity is indicative of the susceptibility of the subject to the cardiovascular condition.

In another embodiment, the present invention provides a method for treating or preventing a neurological condition in a subject. The method includes administering to the subject an effective amount of an agent, wherein the agent regulates the PEMT activity.

In another embodiment, the present invention provides a method for treating or preventing a neurological condition in a subject. The method includes administering to the maternal host of the subject an effective amount of
an agent, wherein the agent regulates the PEMT activity of the maternal host of the subject.

In another embodiment, the present invention provides a method for treating or preventing a neurological condition in a subject. The method includes administering to the subject an effective amount of an agent selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-1 adrenergic receptor agonist, β-2 adrenergic receptor agonist, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

In another embodiment, the present invention provides a method for assessing the susceptibility of a subject to the toxicity of an agent. The method includes determining the level of PEMT activity in the subject, wherein a level of PEMT activity that is lower than a normal PEMT activity is indicative of the susceptibility of the subject to the toxicity of the agent.

In yet another embodiment, the present invention provides a method for assessing the susceptibility of a subject to the toxicity of an agent. The method includes determining the level of CDP-choline activity in the subject, wherein a level of CDP-choline activity that is lower than a normal CDP-choline activity is indicative of the susceptibility of the subject to the toxicity of the agent.

In still another embodiment, the present invention provides a method for assessing the susceptibility of a subject to a condition associated with infertility. The method comprises determining the level of PEMT activity in the subject, wherein a lower than normal PEMT activity is indicative of the susceptibility of the subject to a condition associated with infertility.

In another embodiment, the present invention provides a method for assessing the exposure of a subject to an agent in an environment. The method comprises determining the level of PEMT activity in the subject, wherein a lower than normal PEMT activity is indicative of a higher than normal exposure of the subject to the agent in the environment and wherein the agent is associated with infertility or gender disorder.

In yet another embodiment, the present invention provides a method for assessing a condition in a subject. The method comprises determining the level of PEMT activity in a subject, wherein the level of PEMT activity corresponds to the level of a condition selected from the group consisting of growth
hormone, thyroid activity, eicosanoid production, insulin, and insulin resistance in the subject.

In another embodiment, the present invention provides a method for predicting the effectiveness of an agent for the treatment of a neoplasia. The method comprises determining the level of PEMT activity in a subject in response to the agent, wherein a decrease in the PEMT activity in response to the agent is predictive of the effectiveness of the agent for the treatment of the neoplasia.

In yet another embodiment, the present invention provides a method of increasing milk production in a subject. The method comprises administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases milk production of the subject.

In another embodiment, the present invention provides a method of increasing the lipid content of milk in a subject. The method comprises administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases the lipid content of milk, wherein the lipid is 22:6n3 or 20:4n6.

In another embodiment, the present invention provides a method of increasing the phospholipid content of milk in a subject. The method comprises administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases the phospholipid content of milk.

In yet another embodiment, the present invention provides a method of assessing the susceptibility of a subject to a developmental condition. The method comprises determining the level of PEMT activity in the subject, wherein a lower than normal level of PEMT activity in the subject is indicative of susceptibility of the subject to a developmental condition selected from the group consisting of birth defects, congenital developmental conditions, Microcephaly and mental retardation of uncontrolled phenylketonuria (PKU).

In still another embodiment, the present invention provides a method of assessing the susceptibility of a subject to a developmental condition. The method comprises determining the level of PEMT activity in the maternal host of the subject, wherein a lower than normal level of PEMT activity in the maternal host of the subject is indicative of susceptibility of the subject to a
developmental condition selected from the group consisting of birth defects, congenital developmental conditions, Microcephaly and mental retardation of uncontrolled phenylketonuria (PKU).

In another embodiment, the present invention provides a method of preventing a birth defect of a subject. The method comprises administering to the maternal host of the subject an agent, wherein the agent increases PEMT activity, 22:6n3, or 20:4n6 in the maternal host of the subject.

In yet another embodiment, the present invention provides a method for preventing or treating a birth defect of a subject. The method comprises administering to the subject an agent, wherein the agent increases the level of PEMT activity, 22:6n3, or 20:4n6 in the subject.

In still another embodiment, the present invention provides a method of treating or preventing a condition associated with the PEMT activity in a subject. The method comprises administering to the subject an effective amount of an agent, wherein the agent regulates PEMT activity and wherein the condition associated with the PEMT activity is selected from the group consisting of cirrhosis, skin disorders, neoplasia, infectious diseases, inflammatory diseases, bone density, eicosanoid production, osteoporosis, insulin sensitivity or resistance, diabetes, obesity, renal function loss, autoimmune diseases, and atopic diseases, lipid accumulation in liver, liver steatosis, complications associated with liver transplantation, growth hormone deficiency or overproduction, and thyroid hormone disorders.

SUMMARY OF THE FIGURES

Figures 1A to 1D show the association of PEMT activity with plasma cholesterol ester (CE) and phosphatidylcholine (PC). Figure 1A shows that plasma CE/PC ratio is positively correlated with liver PEMT activity. Figure 1B shows that liver PEMT activity is positively correlated with plasma CE concentrations. Figure 1C shows that liver PEMT activity is positively correlated to some degree with plasma PC concentrations. Figure 1D shows that ACAT2 activity does not predict the CE/PC ratio in plasma.

Figures 2A to 2O show the association of PEMT activity with each major fatty acid in plasma phosphatidylcholine.
DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is based, in part, on the discovery that certain metabolites or metabolic pathways can be used as diagnostic or therapeutic markers. According to the present invention, phosphatidylethanolamine-N-methyltransferase (PEMT) activity and other metabolic activities or markers associated with the PEMT activity are involved in various metabolic processes and disease conditions, and can be used either as markers for diagnosing various conditions or as targets for therapeutic treatment of various disease conditions.

One aspect of the present invention provides that one can regulate the level of a fatty acid by regulating the PEMT activity. The fatty acid regulated by the PEMT activity can be any fatty acid including essential fatty acids in plasma, tissue, and milk. In one embodiment, the fatty acid includes saturated fatty acids, polyunsaturated fatty acids, and highly unsaturated fatty acids. In another embodiment, the fatty acid is 16:0, 18:0, 16:1n7, 18:1n7, 18:2n6, 20:4n6, 20:5n3, or 22:6n3. The fatty acid regulated by the PEMT activity can be free fatty acids or fatty acids in a lipid class. For example, the fatty acid regulated by the PEMT activity can be in phosphatidylcholine or cholesterol esters.

According to the present invention, one can increase the level of a fatty acid in a system, e.g., mammal such as human by regulating the PEMT activity in the system. The level of a fatty acid in a system can be represented by any suitable means, e.g., the absolute or normalized level of a fatty acid such as concentration or composition of the fatty acid. For example, one can increase the level of polyunsaturated fatty acids or highly unsaturated fatty acids by increasing the PEMT activity. In one embodiment, one can increase the level of 18:0, 18:1n7, 20:4n6, 20:5n3, or 22:6n3 by increasing the PEMT activity. In another embodiment, one can increase the level of 16:0, 16:1n7, or 18:2n6 by decreasing the PEMT activity.

According to another aspect of the present invention, one can regulate the level of a fatty acid by directly or indirectly regulating the CDP-choline activity, e.g., regulating any activity associated with CDP-choline pathway and CDP-choline or its isoforms including analogs or derivatives thereof having substantially the same activity as the CDP-choline or CDP-choline pathway.
The fatty acid regulated by the CDP-choline activity can be any fatty acid including essential fatty acids in plasma, tissue, and milk. In one embodiment, the fatty acid includes saturated fatty acids. In another embodiment, the fatty acid is 16:0, 16:1n7, or 18:2n6. The fatty acid regulated by the CDP-choline activity can be free fatty acids or fatty acids in a lipid class. For example, the fatty acid regulated by the CDP-choline activity can be in phosphatidylcholine or cholesterol esters. In one embodiment, one can decrease the level of 16:0, 16:1n7, or 18:2n6 by decreasing the CDP-choline activity.

According to another aspect of the present invention, the density or size of lipoproteins in a system, e.g., mammal such as human can be assessed by determining the PEMT activity, markers of the PEMT activity, or metabolic markers or components that are associated with the PEMT activity in the system. According to the present invention, the PEMT activity usually correlates with the size of lipoproteins while inversely relates to the density of lipoproteins. For example, an increase of the PEMT activity usually corresponds to a decrease of the density and increase of the size of lipoproteins whereas a decrease of the PEMT activity usually corresponds to an increase of the density and decrease of the size of lipoproteins.

Various metabolic markers or components are associated with the PEMT activity and are suitable to be used for assessing the density or size of lipoproteins according to the methods provided by the present invention. For example, fatty acids associated with the PEMT activity can be used to assess the density or size of lipoproteins. In one embodiment, 22:6n3 and 20:4n6 are associated with the PEMT activity, e.g., are markers of the PEMT activity and are used as markers for the density or size of lipoproteins in a system. In another embodiment, the level of homocysteine is associated with the PEMT activity and is used to represent the size or density of lipoproteins in the system, e.g., the level of homocysteine correlates with the density of lipoproteins and inversely relates to the size of lipoproteins in the system.

Lipoprotein component ratios are also associated with the PEMT activity and can be used to represent the density or size of lipoproteins in a system, e.g., mammal such as human. The lipoprotein component ratio of the present invention can be the ratio of any two or more components associated with lipoproteins. Usually components associated with lipoproteins include,
without any limitation, cholesterol ester, free cholesterol, phosphatidylcholine, apoprotein, triacylglyceride, diacylglyceride, and free fatty acids.

In one embodiment, the lipoprotein component ratio of the present invention is the ratio of cholesterol ester to phosphatidylcholine or apoprotein.

In another embodiment, the lipoprotein component ratio of the present invention is the ratio of free cholesterol to apoprotein. In yet another embodiment, the lipoprotein component ratio of the present invention is the ratio of triacylglyceride to phosphatidylcholine. In still another embodiment, the lipoprotein component ratio of the present invention for humans is the ratio of cholesterol ester or free cholesterol to apoprotein.

Usually one or more apoproteins are specifically associated with each particular class of lipoproteins. For example, Apo A-I and Apo A-II are associated with chylomicrons and HDL; Apo B-100 is associated with VLDL, LDL, IDL; Apo B-48 and Apo C-I are associated with chylomicrons; Apo C-II and III are associated with chylomicrons, VLDL, IDL, and HDL; Apo D is associated with HDL and cholesterol ester transfer protein; while Apo E and its multiple isoforms are associated with chylomicron remnants, VLDL, IDL, and HDL.

The lipoprotein component ratio of the present invention can be from any suitable source containing components associated with lipoproteins, e.g., tissue or plasma. In one embodiment, the lipoprotein component ratio is obtained from lipoprotein samples or preparations, e.g., preparations of a particular class or subclass of lipoproteins. In another embodiment, the lipoprotein component ratio is obtained from the preparation of LDL, VLDL, HDL, IDL, chylomicron, chylomicron remnants, or the subclasses thereof. In general, LDL subclasses include large LDL (L3), intermediate LDL (L2), small LDL (L1), VLDL subclasses include large VLDL (V6 and V5), intermediate VLDL (V4 and V3), and small VLDL (V1 and V2), and HDL subclasses include large HDL (H5 and H4), intermediate HDL (H3), and small HDL (H1 and H2).

According to the present invention, the lipoprotein component ratio usually inversely relates to the density of lipoproteins while correlates with the size of lipoproteins. For example, an increase of the lipoprotein component ratio of the present invention usually corresponds to a decrease of the density.
and an increase of the size of lipoproteins whereas a decrease of the lipoprotein component ratio of the present invention usually corresponds to an increase of the density and a decrease of the size of lipoproteins. In one embodiment, the lipoprotein component ratio obtained from a particular class or subclass of lipoprotein inversely relates to the density and correlates to the size of that particular class or subclass of lipoprotein.

According to another aspect of the present invention, the density or size of lipoproteins in a system, e.g., mammal such as human can be regulated by regulating the PEMT activity or metabolic markers or components associated with the PEMT activity in the system. For example, the density of lipoproteins in a system can be increased by decreasing the PEMT activity in the system while the density of lipoproteins in a system can be decreased by increasing the PEMT activity in the system. Additionally, the size of lipoproteins in a system can be decreased by decreasing the PEMT activity in the system while the size of lipoproteins in a system can be increased by increasing the PEMT activity in the system.

In one embodiment, the density or size of lipoproteins in a system is regulated by metabolic markers associated with the PEMT activity. For example, the lipoprotein component ratio of the present invention can be used to regulate the density or size of lipoproteins. The density of lipoproteins in a system can be increased by directly or indirectly decreasing the lipoprotein component ratio of the present invention in the system while the density of lipoproteins in a system can be decreased by directly or indirectly increasing the lipoprotein component ratio of the present invention in the system.

Additionally, the size of lipoproteins in a system can be decreased by directly or indirectly decreasing the lipoprotein component ratio of the present invention in the system while the size of lipoproteins in a system can be increased by directly or indirectly increasing the lipoprotein component ratio of the present invention in the system.

The lipoprotein component ratio can be regulated by any suitable means known to one skilled in the art. According to the present invention, the lipoprotein component ratio can be regulated by regulating the PEMT activity. For example, one can increase the lipoprotein component ratio by directly or
indirectly increasing the PEMT activity and decrease the lipoprotein component ratio by directly or indirectly decreasing the PEMT activity.

In one embodiment, the lipoprotein component ratio is regulated by regulating the PEMT activity relative to the CDP-choline activity. For example, one can regulate the lipoprotein component ratio by directly or indirectly increasing or decreasing the PEMT activity, e.g., PEMT pathway activity as compared to the CDP-choline activity, e.g., CDP-choline pathway activity.

According to another aspect of the present invention, one can prevent or treat cardiovascular conditions in a subject, e.g., mammal such as human by regulating the PEMT activity or metabolic markers or components associated with the PEMT activity in the subject. Various metabolic markers or components are associated with the PEMT activity and are suitable to be used for the prevention and treatment of cardiovascular conditions in a subject.

In one embodiment, the lipoprotein component ratio is regulated individually or in association with the PEMT activity in a subject to prevent or treat cardiovascular conditions in the subject. In another embodiment, one or more fatty acids, e.g., 22:6n3 and 20:4n6 are regulated individually or in association with the PEMT activity in a subject to prevent or treat cardiovascular conditions in the subject.

In general, one can prevent or treat a cardiovascular condition in a subject such as human by administering to the subject one or more agents that directly or indirectly increase the PEMT activity or metabolic markers or components associated with the PEMT activity in the subject. For example, one can administer one or more agents to a subject that increases the PEMT activity, or the lipoprotein component ratio or the level of one or more fatty acids such as 22:6n3 and 20:4n6 either individually or in association with increasing the PEMT activity for the prevention or treatment of cardiovascular conditions. In one embodiment, one can administer one or more agents to a subject that increases the ratio of S-adenosylmethionine to S-adenosylhomocysteine either alone or in association with increasing PEMT activity for the prevention or treatment of cardiovascular conditions.

Various agents are associated with the PEMT activity or metabolic markers or components associated with the PEMT activity and suitable to be used for the prevention or treatment of cardiovascular conditions. According to
the present invention, folic acid, estrogen, vitamin B6, growth hormone, sex
hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine are
capable of increasing the PEMT activity and/or metabolic markers or
components associated with the PEMT activity in a subject and are suitable to
be used for the prevention or treatment of cardiovascular conditions.

According to another aspect of the present invention, one can also assess
the susceptibility of a subject to cardiovascular conditions by monitoring or
determining the PEMT activity or metabolic markers or components associated
with the PEMT activity in the subject, e.g., the lipoprotein component ratio or
one or more fatty acids such as 22:6n3 and 20:4n6. In general, a PEMT activity
of a subject that is lower than the normal level of the PEMT activity is
indicative of the susceptibility of the subject to cardiovascular conditions. In
one embodiment, a lower than normal level of the lipoprotein component ratio,
22:6n3, or 20:4n6 of a subject is indicative of the susceptibility of the subject to
cardi ovascular conditions.

The normal level of the PEMT activity, the lipoprotein component ratio,
or 22:6n3 or 20:4n6 can be obtained by any suitable means known to one skilled
in the art. For example, the normal level can be obtained from one or more
subjects that are known to have no substantial symptoms of any cardiovascular
condition and have no substantial indications of any cardiovascular condition as
measured by other means available in the field. The normal level can be the
level from one normal subject or the mean or average of the levels obtained
from a population of normal subjects.

The cardiovascular condition of a subject that can be prevented or
treated according to the methods provided by the present invention can be any
abnormal, sub-optimal, or pathological condition that is associated with the
function or structure of the cardiovascular system. In one embodiment, the
cardi ovascular condition can be atherosclerosis, coronary artery diseases,
cardiomyopathy, arrhythmia, heart failure, heart attack, stroke, or ischemic heart
diseases. In another embodiment, the cardiovascular condition is
hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, or
hyperglycemia.
According to another aspect of the present invention, one can prevent or treat a neurological condition in a subject, e.g., mammal by regulating the PEMT activity in the subject or maternal host of the subject. For example, one can prevent or treat a neurological condition in a subject by directly or indirectly increasing the PEMT activity in the subject. Alternatively, one can prevent or treat a neurological condition in a subject by administering to the subject one or more agents associated with the PEMT activity, e.g., agents capable of enhancing the PEMT activity in the subject. Such agent includes, without any limitation, folic acid, vitamin B6, growth hormone, sex hormone, β-1, 2, or 3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

In one embodiment, the method is suitable for preventing or treating a neurological condition in an unborn fetus or new born subject by regulating the PEMT activity in the maternal host of the fetus or subject prior to and/or after birth, e.g., via regulating the PEMT activity of the maternal host carrying the fetus and/or providing milk to the subject. According to the present invention, the PEMT activity of the maternal host can be increased in various tissues, e.g., the PEMT activity is increased in liver or mammary gland of the maternal host.

The neurological condition prevented or treated according to the methods provided by the present invention can be any abnormal, sub-optimal, or pathological condition that is associated with the function or structure of the central or peripheral nervous system, e.g., bipolar disorder.

In one embodiment, the neurological condition can be associated with neurodevelopment including, without any limitation, Asperger’s Syndrome, Attention Deficit Hyperactivity Disorder, Autism spectrum disorder, Cerebral Palsy, Dysthymic Disorder, Fragile X Syndrome, Perinatally Acquired HIV Disease, Tourette’s Syndrome, Alternating Hemiplegia of Childhood, Congenital Anomalies, Arnold-Chiari Malformation, Meningocele, Spina Bifida, Dandy Walker Syndrome, vascular malformations, Holoprosencephaly, Hydranencephaly, Rett Syndrome, Hydrocephalus, Polymicrogyria, Pachygyria, Hydranencephaly, Heterotopias, Porencephaly, Megalencephaly, Microcephaly, Agenesis of the corpus callosum, and developmental coordination disorders, e.g., Anencephaly, Cephalocele, Chiari, Spinal dysraphism,
Holoprosencephalies, Corpus callosum agenesis, Facial anomalies, Heterotopias, agryria-pachyria, polymicrogyria, teratomas, and phakomatosis.

In another embodiment, the neurological condition can be associated with neurodegeneration including, without any limitation, Alpers’ disease, Alzheimer’s Disease, Autosomal Dominant Neurodegenerative Disorder, Batten Disease, Cerebral calcinosis, Cockayne Syndrome, corticobasal ganglionic degeneration, Dementia with Lewy Bodies, Lewy Body Variant, Alzheimers Disease, Motor Neuron Disease, Multiple System Atrophy, Parkinson Plus syndrome, Neuronal intranuclear inclusion disease, Olivopontocerebellar Atrophy, Parkinsonian Syndromes, Pick’s disease, Postpoliomyelitis Syndrome, Progressive Supranuclear Palsy, Rett Syndrome, Shy-Drager Syndrome, Tauopathies, Tri-nucleotide repeat diseases, and Tuberous Sclerosis.

In yet another embodiment, the neurological condition in a subject is the neurodevelopment of a human consuming alcohol, e.g., having an alcohol abuse or above average level of alcohol consumption. Alternatively, the neurological condition is fetal alcohol syndrome.

According to another aspect of the present invention, one can assess the susceptibility or sensitivity of a subject to the toxicity or effectiveness of exogenous agents by determining the level of the PEMT activity or CDP-choline activity in the subject. For example, if a subject’s PEMT activity or CDP-choline activity is lower than the normal PEMT or CDP-choline activity, then the subject is susceptible or sensitive to the toxicity or effectiveness of exogenous agents.

In one embodiment, the level of the PEMT activity or CDP-choline activity is used to assess the sensitivity or susceptibility of humans to the toxicity of therapeutic agents. For example, a human having lower than normal level of the PEMT or CDP-choline activity has a lower than normal level of toxicity tolerance and is more susceptible to the toxicity of therapeutics.

In another embodiment, the level of the PEMT activity or CDP-choline activity is used as a marker to assess the effectiveness of therapeutic agents. Usually for various conditions the level of the PEMT activity corresponds to or is the marker of the conditions, thus can be used to monitor or assess the effectiveness of the therapeutic agents for the treatment of the conditions. For example, the level of the PEMT activity can be used to monitor the level of
growth hormone, thyroid activity, insulin sensitivity, and lipid accumulation in
liver and the effectiveness of therapeutic agents for the treatment of growth
hormone deficiency or overproduction, hypothyroid or hyperthyroid activity,
insulin sensitivity or resistance, lipid accumulation in liver, and any conditions
associated therewith.

In yet another embodiment, the level of the PEMT activity in a subject
in response to a therapeutic agent is used as a marker to predict the effectiveness
of the therapeutic agents to the subject. In general, the effectiveness of an agent
in inhibiting a neoplastic growth in a subject, e.g., mammal such as human
corresponds to the effectiveness of such agent in decreasing PEMT activity in
the subject. Thus the level of a decrease in PEMT activity in a subject in
response to an agent can be used as a marker to predict the effectiveness of the
agent in treating the neoplastic growth in the subject.

According to the present invention, a decrease in the PEMT activity in a
subject can be used to assess the effectiveness of any therapeutic agent suitable
for the treatment of neoplastic growth, especially if the neoplastic growth is
associated with a hormone level of the subject and the agent affects such
hormone level in the subject. For example, the agent can be a PPAR agonist,
nuclear hormone receptor agonist, or the analog or derivative thereof. The
nuclear hormone receptor can be any including, without any limitation the
receptors for thyroid hormone, estrogen, and retinoid. Usually the agent is used
to treat any neoplastic growth including cancer or tumor, especially an abnormal
growth that is hormone responsive, e.g., PPAR responsive colon cancer, thyroid
cancer, pancreatic cancer, esophageal squamous carcinoma, and prostate cancer.

In still another embodiment, the level of the PEMT activity or CDP-
choline activity is used to assess the sensitivity or susceptibility of a subject,
e.g., mammals such as humans to the toxicity of an environment or agents
contained in an environment, thus the subject's predisposition to abnormal
conditions associated with the toxicity or agents contained in the environment.

According to the present invention, a subject, e.g., mammal such as
human having lower than normal level of the PEMT or CDP-choline activity
usually has a higher than normal level of sensitivity towards the toxicity of an
environment or the toxicity of one or more agents contained in an environment.
For example, a subject having lower than normal level of the PEMT activity can
be indicative of higher than normal exposure to agents associated with decreased reproduction and gender disorders, e.g., anti-estrogen or dioxins, thus indicative of the subject’s predisposition to conditions associated with reproduction and gender, e.g., decreased sperm motility and fertility, gender confusion, feminization, or gender skewing.

According to another aspect of the present invention, one can assess the susceptibility, predisposition, or likelihood of a subject to a developmental condition by determining the level of the PEMT activity in the subject or the subject has been exposed to prior to and/or after birth, e.g., the level of the PEMT activity in the maternal host of a subject. For example, a subject or the maternal host of a subject having a lower than normal level of the PEMT activity is indicative of the susceptibility or predisposition of the subject to developmental conditions.

According to yet another aspect of the present invention, one can prevent or treat a developmental condition in a subject, e.g., mammal such as human by regulating the PEMT activity or metabolic markers or components associated with the PEMT activity in the subject or maternal host of the subject. For example, various developmental conditions can be prevented or treated by increasing the PEMT activity or metabolic markers or components associated with the PEMT activity of the subject or the maternal host of the subject.

In one embodiment, the subject is an unborn fetus and the PEMT activity of the maternal host of the fetus is increased in various tissues, e.g., liver and mammary gland. In another embodiment, the subject is newly born and intakes milk from the maternal host where the PEMT activity of the maternal host of the newly born subject is increased in various tissues, e.g., liver and mammary gland. In yet another embodiment, the subject is newly born and intakes milk from the maternal host where one or more metabolic markers or components in the milk content is increased, e.g., polyunsaturated fatty acid, 22:6n3, or 20:4n6 is increased either alone or in association with increasing the PEMT activity. In still another embodiment, the subject is newly born and the PEMT activity, metabolic markers or components associated with the PEMT activity of the subject is increased, e.g., by supplementing the intakes of the subject with PEMT increasing agents, polyunsaturated fatty acid, 22:6n3, or 20:4n6.
According to the present invention, a developmental condition can be any condition associated with the development of a subject, e.g., mammal prior to and/or after birth. In one embodiment, the developmental condition is one or more birth defects or congenital developmental conditions including, without limitation, conditions associated with immunological functions, psychomotor development such as cognitive neural development, motor skills, cognitive functioning, and mental development, visual and stereo acuity, probiotics actions, and atopic diseases such as eczema, atopic dermatitis, asthma, and allergic rhinitis. In another embodiment, the developmental condition of a subject is one or more congenital developmental conditions associated with the diabetic condition of the maternal host of the subject. In yet another embodiment, the developmental condition of a subject is Microcephaly and mental retardation of uncontrolled phenylketonuria (PKU).

According to another aspect of the present invention, the PEMT activity can be generally used as a marker for various biological conditions associated with the PEMT activity in a subject. For example, the PEMT activity can be used as a marker to monitor the level of growth hormone, thyroid condition, eicosanoid production, the level of 20:4n6 or 22:6n3, or insulin level or sensitivity. In one embodiment, the PEMT activity in a subject corresponds to the level of growth hormone, eicosanoid production, thyroid condition, and the level of 20:4n6 and 22:6n3 in the subject. In another embodiment, the PEMT activity in a subject corresponds to the insulin level in the subject while a higher than normal level of the PEMT activity in a subject is indicative of decreased insulin sensitivity and increased insulin resistance in the subject.

According to yet another aspect of the present invention, one can generally treat a condition associated with the PEMT activity in a subject, e.g., mammal by regulating the PEMT activity or metabolic markers or components associated with the PEMT activity in the subject. Various conditions are associated with the PEMT activity in a subject and can be treated by regulating the PEMT activity in the subject. In one embodiment, regulating the PEMT activity or metabolic markers or components associated with the PEMT activity can be used to treat cirrhosis, skin disorders, neoplasia, infectious diseases, inflammatory diseases, e.g., rheumatoid arthritis, lupus, chronic glomerular diseases, chronic bowel inflammation, and nephropathy, bone density,
Eicosanoid production, osteoporosis, insulin sensitivity or resistance, diabetes, obesity, renal function loss, autoimmune diseases, *e.g.*, systemic lupus erythematosus, and atopic diseases, *e.g.*, eczema, atopic dermatitis, asthma, and allergic rhinitis.

In another embodiment, regulating the PEMT activity or metabolic markers or components associated with the PEMT activity can be used to treat complications associated with liver transplantation, lipid accumulation in liver, liver steatosis, and conditions associated therewith, *e.g.*, diabetes, obesity, alcoholism, drug abuse, hepatitis, and hormone imbalances including those involved in growth hormone deficiency or overproduction and thyroid hormone conditions.

In yet another embodiment, increasing the PEMT activity or metabolic markers or components associated with the PEMT activity can be used to treat conditions associated with the PEMT activity in a subject including, without any limitation, skin disorders, infectious diseases, rheumatoid arthritis, osteoporosis, atopic diseases, cirrhosis, renal functional loss, autoimmune diseases, lipid accumulation in liver and conditions associated therewith, complications associated with liver transplantation, insulin resistance and complications associated therewith, growth hormone deficiency or excess, thyroid hormone and to increase the level of eicosanoid production, bone density, milk production, or the phospholipid or polyunsaturated fatty acid content of milk production in the subject.

For example, increasing the PEMT activity or the level of 20:4n6 or 22:6n3 can be used to treat rheumatoid arthritis and asthma, especially with suppressed production of pro-inflammatory cytokines, renal functional loss in patients with IgA nephropathy, autoimmune diseases such as spontaneous autoantibody-mediated systemic lupus erythematosus, and complications associated with liver transplantation especially under the treatment of cyclosporin A.

In still another embodiment, decreasing the PEMT activity can be used to treat conditions associated with the PEMT activity including, without any limitation, neoplasia (except hepatocarcinoma), chronic bowel inflammation, and increasing insulin sensitivity or decreasing insulin resistance.
According to the present invention, the PEMT activity as used in any aspect of the present invention can be any activity associated with PEMT pathway or PEMT activity and its isoforms including analogs or derivatives thereof having substantially the same activity as PEMT. The PEMT gene usually encodes two enzymatic isoforms, PEMT1 and PEMT2. PEMT1 activity is usually present in the endoplasmic reticulum and is likely the isoform involved in lipoprotein export. PEMT2 activity is usually found in the mitochondria.

Regulation of the PEMT activity as used in any aspect of the present invention can be direct or indirect regulation and in general can be directed to the PEMT activity in plasma or various tissues, e.g., liver, intestine, heart, brain, and mammary tissue. One can regulate the PEMT activity by using any suitable means known to one skilled in the art. For example, one can regulate the PEMT activity by directly or indirectly regulating the expression level of PEMT gene, the translation level of PEMT gene, or the stability of PEMT and its isoforms.

Alternatively, one can regulate the PEMT activity by using agents that directly or indirectly regulate the PEMT activity, e.g., PEMT inhibitors, antagonists, or agonists. In one embodiment, the PEMT activity is regulated by regulating the PEMT activity relative to CDP-choline activity. In another embodiment, according to the present invention, one can increase the PEMT activity in a subject, e.g., mammal such as human by administering to the subject an agent including, without limitation, folic acid, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine. In yet another embodiment, according to the present invention, one can decrease the PEMT activity in a subject, e.g., mammal such as human by administering to the subject an agent including, without limitation, fibrate, dioxins, anti-estrogen agents, and PPAR-α agonists.

Any agents used for the therapeutic methods provided by the present invention can be provided as a composition including one or more other non-active ingredients, e.g., ingredients that do not interfere with the function of the active ingredients. For example, the composition containing one or more agents of the present invention can include a suitable carrier or be combined with other therapeutic agents.
A suitable carrier can be an aqueous carrier including any safe and effective materials for use in the compositions of the present invention. In one embodiment, an aqueous carrier is used for the compositions of the present invention in oral formations and includes, without limitation, thickening materials, humectants, water, buffering agents, abrasive polishing materials, surfactants, titanium dioxide, flavor system, sweetening agents, coloring agents, and mixtures thereof.

A suitable carrier can also be a pharmaceutically acceptable carrier that is well known to those in the art. Such carriers include, without limitation, large, slowly metabolized macromolecules, e.g., proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and inactive virus particles.

Pharmaceutically acceptable salts can also be used in the composition, for example, mineral salts such as sodium or stannous fluorides, or sulfates, as well as the salts of organic acids such as acetates, propionates, carbonates, malonates, or benzoates. The composition can also contain liquids, e.g., water, saline, glycerol, and ethanol, as well as substances, e.g., wetting agents, emulsifying agents, or pH buffering agents.

In generally, an effective amount of the agents of the present invention to be administered can be determined on a case-by-case basis. Factors to be considered usually include age, body weight, stage of the condition, other disease conditions, duration of the treatment, and the response to the initial treatment.

Typically, the agents of the present invention are prepared as a topical or an injectable, either as a liquid solution or suspension. However, solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The composition can also be formulated into an enteric-coated tablet or gel capsule according to known methods in the art.

The agents of the present invention may be administered in any way which is medically acceptable which may depend on the condition or injury being treated. Possible administration routes include injections, by parenteral routes such as intravascular, intravenous, intraepidural or others, as well as oral, nasal, ophthalmic, rectal, vaginal, topical, or pulmonary, e.g., by inhalation. The compositions may also be directly applied to tissue surfaces. Sustained
release, pH dependent release, or other specific chemical or environmental condition mediated release administration is also specifically included in the invention, by such means as depot injections or erodible implants.

EXAMPLES

The following examples are intended to illustrate but not to limit the invention in any manner, shape, or form, either explicitly or implicitly. While they are typical of those that might be used, other procedures, methodologies, or techniques known to those skilled in the art may alternatively be used.

Example I. Lipid activity and Cardiovascular Diseases

Factors that influence both production of lipoproteins and the size and density distribution of lipoproteins play a major role in the pathogenesis of cardiovascular disease.

Very-low density lipoproteins (VLDL) are triacylglycerol-rich particles that are assembled from lipids in the liver. The major factor regulating hepatic very low-density lipoprotein (VLDL) assembly and secretion is the availability of lipids, such as PC, the major phosphopholipid component of VLDL. The active synthesis of PC is required for VLDL secretion from rat hepatocytes (Yao ZM, Vance DE 1988. J Biol Chem 263: 2998–3004.). Their studies with rat hepatocytes suggested that hepatic secretion of VLDL, but not high-density lipoprotein (HD), required active PC biosynthesis. Their study showed that the inhibitory effect of choline deficiency on VLDL secretion can be compensated for by the methylation of PE (the PEMT pathway).

Yao and Vance (1990. Biochem Cell Biol 68: 552–558.) also observed that in hepatocytes of rats deficient in choline, decreased VLDL secretion was a consequence of severely reduced PC synthesis. Plasma VLDL levels were reduced in choline-deficient rats, but the concentration of plasma HDL was not affected, supporting the hypothesis that choline deficiency causes reduction of VLDL, but not HDL, in plasma as a consequence of impaired hepatic VLDL secretion.

Yao and Vance (1989. J Biol Chem 264: 11373–11380) also investigated the head group specificity of phospholipid synthesis required for lipoprotein secretion in cultured hepatocytes isolated from choline-deficient
rats. Their results suggested that the choline head group moiety of PC is specifically required for normal VLDL secretion and cannot be replaced with ethanolamine, monomethylethanolamine or dimethylethanolamine. Thus, the production of PC by either the CDP-choline pathway or the PEMT pathway is essential for normal lipoprotein assembly and export from the liver.

Vance et al. (1998. Curr Opin Lipidol 9: 125–130.) studied the role that PC biosynthesis plays in the assembly and secretion of lipoproteins. A specific inhibitor of cellular transmethylation, 3-deazaadenosine, was incubated with rat hepatocytes, and PC synthesis via methylation of PE derived from ethanolamine was inhibited more than 95%. However, secretion of the apoproteins into VLDL, LDL, HDL fractions or a fraction with density greater than 1.18 g/ml was not affected, nor was the amount of PC secreted into the medium or into VLDL or HDL affected. The amount of PE in the cells doubled, and the amount secreted into the medium increased 70%. The results indicated that the synthesis of PC from ethanolamine is not required for lipoprotein secretion by rat hepatocytes.

However, Nishimaki-Mogami et al. (2002. J Lipid Res 43: 1035–1045.) studied the role of PC synthesis via the PE methylation pathway in the secretion of VLDL by cultured rat hepatocytes by determining the effects of inhibitors of PE methylation, (bezafibrate and clofibric acid, both of which are hypolipidemic agents).

These investigators found that bezafibrate elicited a 60% reduction of TG secretion and thus VLDL export. However, their results indicated that bezafibrate increases the movement of apoB-48 from the membrane to the lumen. The PE methylation pathway was rapidly inactivated by methionine depletion. Reduced PE methylation impairs the late stage of assembly for the addition of bulk core lipids into VLDL-apoB-48. It was thus suggested that PE methylation is at least very important for apoB-48 synthesis and HDL-B-48 formation. Reduced TG secretion by bezafibrate requires PC synthesis via the PE methylation pathway. The two pathways for phosphatidylcholine biosynthesis may thus play different roles in the production of VLDL.
PEMT activity and lipoprotein particles

Inventors of the present invention have discovered, in part, that the activity of the PEMT pathway and the activity of PEMT relative to the activity of the CDP-choline pathway can be critical for determining the size and composition of lipoprotein particles. Using a surrogate marker for lipoprotein size and density (the ratio of the concentration of plasma CE and the concentration of plasma PC), the percentage contribution of PEMT to total plasma phosphatidylcholine concentrations (as assessed by our PEMT equations as described in the U.S. Patent Application No. 60/424,949) was shown to positively correlate with the CE/PC ratio (Fig. 1). Thus, changes in liver PEMT activity (or its activity relative to the CDP-choline pathway) cause changes in the size and density of lipoprotein particles in plasma and consequently can modulate phenotypes associated with cardiovascular disease.

PEMT activity and fatty acid level

Six groups of mice including homozygous deletion, homozygous control: choline-deficient and choline-supplemented, and control diets were used in all of the graphs in Figures 2A to 2O.

The relative activity of the PEMT and CDP-choline pathways for producing phosphatidylcholine were manipulated by (1) comparing mice carrying a homozygous deletion of PEMT with control mice, and (2) treating these groups of mice with choline-deficient, control and choline-supplemented diets. The relative proportion of liver PC produced by the PEMT was estimated using the equation described above and plotted on the x-axis of the graphs in Figure 2.

The effect of PEMT activity on plasma PC fatty acid composition was then assessed by plotting the mole percentage composition of each major fatty acid in plasma PC against the assessment of liver PEMT activity. Regression results were significant at an $\alpha < 0.05$ if the $r^2$ value was greater than 0.24.

Prominent differences include a negative effect of PEMT on the 16:0, 16:1n7, 18:2n6, and total saturated fatty acid composition of plasma PC. PEMT had a significant positive effect on the 18:0, 18:1n7, 20:4n6, 22:6n3 and total PUFA content of plasma PC. Particularly relevant to neurodevelopment and other phenotypes related to essential fatty acid metabolism (stroke, CVD, diabetes, etc.).
was the strong positive effect of PEMT on the arachidonic (20:4n6) and docosahexaenoic acid (22:6n3) composition of PC. These fatty acids are essential and involved in many physiological processes including neurodevelopment and neural function, cell signaling, cardiac and mitochondrial function, and are correlated with a number of pathologies.

As phosphatidylcholine is the primary source of 20:4n6 and 22:6n3 in plasma (cholesterol ester is another reasonably rich source, but the 20:4n6 and 22:6n3 content of plasma CE is derived directly from plasma PC), PEMT activity determines the plasma concentration of these essential fatty acids, and thus, controls the distribution of these essential fatty acids to tissues including brain and heart.

**Folic acid**

Folic acid increases the ratio of S-adenosylmethionine to S-adenosylhomocysteine by donating its methyl groups to S-adenosylhomocysteine. In turn, the increased ratio of S-adenosylmethionine to S-adenosylhomocysteine can drive methylation reactions in the cell that use S-adenosylmethionine as a methyl donor, including PEMT activity. Hence, factors that increase this ratio can increase the activity of PEMT, decrease the plasma CE/PC ratio and decrease the susceptibility to CVD disease.

It has not been conclusively demonstrated whether plasma homocysteine concentrations are causative of increased cardiovascular diseases or whether homocysteine is a marker for other metabolisms that cause cardiovascular disease. Elevated homocysteine concentrations are a marker of decreased methylation capacity and thus, a marker of decreased PEMT activity. Hence, while increased plasma homocysteine concentrations may have direct effects on cardiovascular disease, it also serves as a marker for an altered lipoprotein size and density profile as a result of decreased PEMT activity.

To estimate the size and density of lipoprotein particles, the ratio of plasma cholesterol ester or triacylglyceride to plasma phosphatidylcholine in plasma was calculated:

\[
\text{Estimated Particle Size/Density 1} = \frac{\text{PLACE}_\text{tot n mol per g}}{\text{PLAPC}_\text{tot n mol per g}}
\]
Estimated Particle Size/Density 2 = (PLATGtot_n_mol_per_g) / (PLAPCtot_n_mol_per_g)

**Example II. Lipid Activity and Neurological Conditions**

There is a strong correlation between liver PEMT activity and plasma phosphatidylcholine 20:4n6 and 22:6n3 concentrations (Fig. 2). These fatty acids are essential for neurodevelopment and other neurological processes. Thus, agents that affect PEMT activity stand to affect neurodevelopment, etc. This is interesting in light of the fact that folate, known to decrease the rate of birth defects, up-regulates PEMT activity and alcohol, known to increase birth defects, down-regulates PEMT activity.

**Alcohol**

The web site for the Center for Disease Control states that “Children exposed to alcohol during fetal development can suffer a wide array of disorders, from subtle changes in I.Q. to profound mental retardation. They can also suffer growth retardation in varying degrees and be born with birth defects of major organ systems.” [http://www.cdc.gov/ncbddd/fas/default.htm](http://www.cdc.gov/ncbddd/fas/default.htm).


As shown in Figure 2, PEMT activity is essential for mobilizing PC containing the fatty acids 20:4n6 and 22:6n3 out of tissues into plasma. Thus, PEMT inhibition and the resulting depletion of essential fatty acids from plasma and peripheral tissues (including brain) can be the metabolic basis for fetal alcohol syndrome.

**Vitamin B6 deficiency**

The content of PC in liver microsomes from rats fed a vitamin B6-deficient 70% casein diet for 5 weeks was decreased considerably in PC
content (She et al., 1995). The hepatic level of the PEMT co-substrate, S-adenosylmethionine, decreased about one-third, but the level of the inhibitory metabolite, S-adenosylhomocysteine, was elevated. This study demonstrated that vitamin B6 deficiency modified methionine metabolism and decreased choline utilization, and thus indirectly affected the biosynthesis of PC in liver microsomes.

Folic acid

Folic acid increases the ratio of S-adenosylmethionine to S-adenosylhomocysteine by donating its methyl groups to S-adenosylhomocysteine. In turn, the increased ratio of S-adenosylmethionine to S-adenosylhomocysteine can drive methylation reactions in the cell that use S-adenosylmethionine as a methyl donor, including PEMT activity. Thus, folate can increase PEMT activity and increase the concentration of 20:4n6 and 22:6n3 in the maternal bloodstream as well as in milk lipids.

Diabetes and Insulin


Response to Estrogen-phytoestrogens and anti-estrogens

In general, estrogen increases PEMT activity, but the amount of the increase varies by individual. These individual differences in the responsiveness of PEMT to estrogen can be used to explain why some women who take estrogen or birth control pills develop adverse clinical disease (CVD, osteoporosis, cancer, etc) and others do not. The same holds for the differential response of women during and after menopause (when hormone levels decrease) and the development of adverse clinical disease. PEMT activity can be directly related to the development of these diseases and/or a marker for their development. Therefore, measuring PEMT activity can be used to predict which individuals are at risk to develop the related diseases. In particular measuring PEMT activity in response to estrogen can be used to predict who is
at risk in developing the related diseases. This can be used as a screen before starting hormone therapy or at menopause to recommend hormone therapy.

In addition, antiestrogens, such as tamoxifen, are used to treat breast cancer as well as other cancers. Some women respond better than others.

Measurement of PEMT activity in response to cancer treatment can be used as an early marker for response to treatment. The cancer treatment can include treatment for breast cancer, uterine, and other cancers.

Antiestrogens and fibrates decrease PEMT. Antiestrogens and fibrates in the environment have been linked to an increase in birth defects in animals.

Therefore, PEMT activity could be used to assess the exposure of a population to PPARα (fibrates) or anti-estrogens and the population’s potential for birth defects. This could be done on an individual basis as well.

Antiestrogens decrease sperm motility and male fertility. Measurement of PEMT activity in males could be used as a measure of population exposure to antiestrogens and the potential for infertility. This could be done on an individual basis as well. The population can be both humans and any other species, such as frogs, which may be affected by antiestrogens and xenoestrogens.

In frogs, fish, and nematodes, treatment of animals with anti-estrogens leads to gender confusion, feminization, or gender skewing. Other species such as polar bears have become hemaphrodites on exposure to environmental xenoestrogens. It is believed that exposure to environmental estrogens in early post-natal life can lead to altered development of the reproductive system (this was shown in mice). Exposure of children to environmental estrogens is thought to lead to early onset puberty in females. Again, measurement of PEMT activity in a population could be used to predict environmental exposure to estrogens and the potential for early onset puberty in females.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.
What is claimed is:

1. A method for regulating the level of a fatty acid in a system comprising regulating the phosphatidylethanolamine-N-methyltransferase (PEMT) activity in the system whereby regulating the level of a fatty acid in the system, wherein the fatty acid is selected from the group consisting of 16:0, 18:0, 16:1n7, 18:1n7, 18:2n6, 20:4n6, 20:5n3, 22:6n3, saturated fatty acids, polyunsaturated fatty acids, and highly unsaturated fatty acids.

2. The method of claim 1, wherein the level of the fatty acid is increased by increasing the PEMT activity and wherein the fatty acid is selected from the group consisting of 18:0, 18:1n7, 20:4n6, 20:5n3, 22:6n3, polyunsaturated fatty acids, and highly unsaturated fatty acids.

3. The method of claim 1, wherein the level of the fatty acid is increased by increasing the PEMT activity relative to CDP-choline activity and wherein the fatty acid is selected from the group consisting of 18:0, 18:1n7, 20:4n6, 20:5n3, 22:6n3, polyunsaturated fatty acids, and highly unsaturated fatty acids.

4. The method of claim 1, wherein the level of the fatty acid is increased by decreasing the PEMT activity and wherein the fatty acid is selected from the group consisting of 16:0, 16:1n7, 18:2n6, and saturated fatty acids.

5. The method of claim 1, wherein the level of the fatty acid is increased by decreasing the PEMT activity relative to CDP-choline activity and wherein the fatty acid is selected from the group consisting of 16:0, 16:1n7, 18:2n6, and saturated fatty acids.

6. The method of claim 1, wherein the fatty acid is in plasma.

7. The method of claim 1, wherein the fatty acid is in a tissue.
8. The method of claim 1, wherein the fatty acid is in milk.

9. The method of claim 1, wherein the fatty acid is in a lipid class.

10. The method of claim 1, wherein the fatty acid is in phosphatidylcholine or cholesterol esters.

11. A method for decreasing the level of a fatty acid in a system comprising decreasing the CDP-choline activity in the system whereby decreasing the level of a fatty acid in the system, wherein the fatty acid is selected from the group consisting of 16:0, 16:1n7, 18:2n6, and saturated fatty acids.

12. The method of claim 11, wherein the fatty acid is in plasma.

13. The method of claim 11, wherein the fatty acid is in a tissue.

14. The method of claim 11, wherein the fatty acid is in milk.

15. A method for increasing the PEMT activity in a subject comprising administering to the subject an effective amount of an agent selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

16. The method of claim 15, wherein the PEMT activity is in liver, intestine, heart, brain, or mammary tissue.

17. A method for regulating a lipoprotein component ratio in a system comprising regulating the PEMT activity in the system whereby regulating the lipoprotein component ratio in the system, wherein the lipoprotein component ratio is selected from the group consisting of
cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine.

18. The method of claim 17, wherein the lipoprotein component ratio is in plasma.

19. The method of claim 17, wherein the lipoprotein component ratio is in a lipoprotein.

20. The method of claim 17, wherein the lipoprotein component ratio is in a lipoprotein selected from the group consisting of LDL, VLDL, HDL, IDL, chylomicron remnants, HDL subclasses, and chylomicron.

21. The method of claim 17, wherein the PEMT activity is relative to the CDP-choline activity.

22. The method of claim 17, wherein the PEMT activity is in liver, intestine, heart, brain, or mammary tissue.

23. The method of claim 17, wherein the lipoprotein component ratio is increased by increasing the PEMT activity.

24. The method of claim 17, wherein the lipoprotein component ratio is increased by increasing the PEMT activity relative to the CDP-choline activity.

25. The method of claim 17, wherein the lipoprotein component ratio is decreased by decreasing the PEMT activity.

26. The method of claim 17, wherein the lipoprotein component ratio is decreased by decreasing the PEMT activity relative to the CDP-choline activity.
27. The method of claim 17, wherein the lipoprotein component ratio is cholesterol ester to apoprotein in human.

28. The method of claim 17, wherein the lipoprotein component ratio is free cholesterol ester to apoprotein in human.

29. A method of regulating the density or size of a lipoprotein in a system comprising regulating a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system whereby regulating the density or size of a lipoprotein in the system.

30. The method of claim 29, wherein the lipoprotein component ratio is in plasma.

31. The method of claim 29, wherein the lipoprotein component ratio is in a lipoprotein.

32. The method of claim 29, wherein the lipoprotein component ratio is cholesterol ester to apoprotein in human.

33. The method of claim 29, wherein the lipoprotein component ratio is free cholesterol to phosphatidylcholine in human.

34. The method of claim 29, wherein the lipoprotein component ratio is in a lipoprotein selected from the group consisting of LDL, VLDL, HDL, IDL, chylomicron remnants, HDL subclasses, and chylomicron.

35. The method of claim 29, wherein the density of a lipoprotein is increased by decreasing the lipoprotein component ratio.

36. The method of claim 29, wherein the density of a lipoprotein is decreased by increasing the lipoprotein component ratio.
37. The method of claim 29, wherein the size of a lipoprotein is decreased by decreasing the lipoprotein component ratio.

38. The method of claim 29, wherein the size of a lipoprotein is increased by increasing the lipoprotein component ratio.

39. The method of claim 29, wherein the density of a lipoprotein is increased by decreasing the lipoprotein component ratio via decreasing the PEMT activity.

40. The method of claim 29, wherein the density of a lipoprotein is increased by decreasing the lipoprotein component ratio via decreasing the PEMT activity relative to the CDP-choline activity.

41. The method of claim 29, wherein the density of a lipoprotein is decreased by increasing the lipoprotein component ratio via increasing the PEMT activity.

42. The method of claim 29, wherein the density of a lipoprotein is decreased by increasing the lipoprotein component ratio via increasing the PEMT activity relative to the CDP-choline activity.

43. A method for regulating the density or size of a lipoprotein in the system comprising regulating the PEMT activity in the system whereby regulating the density or size of a lipoprotein in the system.

44. The method of claim 43, wherein the PEMT activity is in liver, intestine, heart, brain, or mammary tissue.

45. The method of claim 43, wherein the density of a lipoprotein is increased by decreasing the PEMT activity.
46. The method of claim 43, wherein the density of a lipoprotein is increased by decreasing the PEMT activity relative to the CDP-choline activity.

47. The method of claim 43, wherein the size of a lipoprotein is decreased by decreasing the PEMT activity.

48. The method of claim 43, wherein the density of a lipoprotein is decreased by increasing the PEMT activity.

49. The method of claim 43, wherein the density of a lipoprotein is decreased by increasing the PEMT activity relative to the CDP-choline activity.

50. The method of claim 43, wherein the size of a lipoprotein is increased by increasing the PEMT activity.

51. A method of assessing the density of a lipoprotein in a system comprising determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system wherein the lipoprotein component ratio is inversely related to the density of the lipoprotein in the system.

52. A method of assessing the size of a lipoprotein in a system comprising determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, and triacylglyceride to phosphatidylcholine in the system wherein the lipoprotein component ratio correlates with the size of the lipoprotein in the system.

53. The method of claim 51, wherein the lipoprotein component ratio is in plasma.
54. The method of claim 51, wherein the lipoprotein component ratio is cholesterol ester to apoprotein in human.

55. The method of claim 51, wherein the lipoprotein component ratio is free cholesterol to phosphatidylcholine in human.

56. A method of assessing the density of a lipoprotein in a system comprising determining the PEMT activity in the system, wherein the PEMT activity is inversely related to the density of the lipoprotein in the system.

57. A method of assessing the size of a lipoprotein in a system comprising determining the PEMT activity in the system, wherein the PEMT activity correlates with the size of the lipoprotein in the system.

58. A method of assessing the density of a lipoprotein in a system comprising determining the homocysteine level of the system, wherein the homocysteine level correlates with the density of the lipoprotein in the system.

59. A method of assessing the size of a lipoprotein in a system comprising determining the homocysteine level of the system, wherein the homocysteine level is inversely related to the size of the lipoprotein in the system.

60. A method for treating or preventing a cardiovascular condition comprising administering to a subject in need of such treatment an agent, wherein the agent regulates a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to apoprotein, triacylglyceride to phosphatidylcholine, and S-adenosylmethionine to S-adenosylhomocysteine.
61. The method of claim 60, wherein the agent regulates the lipoprotein component ratio in plasma.

62. The method of claim 60, wherein the subject is human and the lipoprotein component ratio is cholesterol ester to apoprotein or free cholesterol to phosphatidylcholine.

63. The method of claim 60, wherein the lipoprotein component ratio is in a lipoprotein.

64. The method of claim 60, wherein the lipoprotein component ratio is in a lipoprotein selected from the group consisting of LDL, VLDL, HDL, IDL, chylomicron remnants HDL subclasses, and chylomicron.

65. The method of claim 60, wherein the agent regulates the lipoprotein component ratio via regulating the PEMT activity.

66. The method of claim 60, wherein the agent regulates the lipoprotein component ratio via regulating the PEMT activity relative to the CDP-choline activity.

67. The method of claim 60, wherein the agent increases the lipoprotein component ratio.

68. The method of claim 60, wherein the agent increases the lipoprotein component ratio via increasing the PEMT activity.

69. The method of claim 60, wherein the agent increases the lipoprotein component ratio via increasing the PEMT activity relative to the CDP-choline activity.

70. The method of claim 60, wherein the cardiovascular condition is atherosclerosis.
71. The method of claim 60, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery heart diseases, and heart attack.

72. A method for treating or preventing a cardiovascular condition comprising administering to a subject in need of such treatment an agent, wherein the agent regulates the PEMT activity.

73. The method of claim 72, wherein the PEMT activity is in liver, heart, intestine, or placenta in the maternal host of the subject.

74. The method of claim 72, wherein the agent increases the PEMT activity.

75. The method of claim 72, wherein the agent increases the PEMT activity relative to the CDP-choline activity.

76. The method of claim 72, wherein the agent is estrogen, folic acid, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

77. The method of claim 72, wherein the cardiovascular condition is atherosclerosis.

78. The method of claim 72, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery diseases, and heart attack.

79. A method for treating or preventing a cardiovascular condition comprising administering to a subject in need of such treatment an
effective amount of estrogen, folic acid, vitamin B6, growth hormone, sex hormone, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

80. The method of claim 79, wherein the cardiovascular condition is atherosclerosis.

81. The method of claim 79, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery diseases, and heart attack.

82. A method for treating or preventing a cardiovascular condition comprising administering to a subject in need of such treatment an effective amount of an agent, wherein the agent regulates the level of 22:6n3 or 20:4n6.

83. The method of claim 82, wherein the level of 22:6n3 or 20:4n6 is in phosphatidylcholine.

84. The method of claim 82, wherein the level of 22:6n3 or 20:4n6 is in cholesterol ester.

85. The method of claim 82, wherein the agent increases the level of 22:6n3 or 20:4n6.

86. The method of claim 82, wherein the cardiovascular condition is atherosclerosis.

87. The method of claim 82, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension,
hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery diseases, and heart attack.

88. A method for assessing the susceptibility of a subject to a cardiovascular condition comprising determining a lipoprotein component ratio selected from the group consisting of cholesterol ester to phosphatidylcholine, cholesterol ester to apoprotein, free cholesterol to phosphatidylcholine, triacylglyceride to phosphatidylcholine in the subject, wherein a lipoprotein component ratio that is higher than a normal lipoprotein component ratio is indicative of the susceptibility of the subject to the cardiovascular condition.

89. The method of claim 88, wherein the lipoprotein component ratio is in plasma.

90. The method of claim 88, wherein the lipoprotein component ratio is in a lipoprotein.

91. The method of claim 88, wherein the lipoprotein component ratio is in a lipoprotein selected from the group consisting of LDL, VLDL, HDL, IDL, chylomicron remnants, HDL subclasses, and chylomicron.

92. The method of claim 88, wherein the cardiovascular condition is atherosclerosis.

93. The method of claim 88, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery diseases, and heart attack.

94. A method for assessing the susceptibility of a subject to a cardiovascular condition comprising determining the PEMT activity in the subject, wherein a PEMT activity that is lower than a normal PEMT activity is
indicative of the susceptibility of the subject to the cardiovascular condition.

95. The method of claim 94, wherein the PEMT activity is in liver or plasma.

96. The method of claim 94, wherein the PEMT activity is relative to CDP-choline activity.

97. The method of claim 94, wherein the cardiovascular condition is atherosclerosis.

98. The method of claim 94, wherein the cardiovascular condition is selected from the group consisting of hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hypertension, hyperglycemia, stroke, ischemic heart diseases, cardiomyopathy, arrhythmia, coronary artery diseases and heart attack.

99. A method for treating or preventing a neurological condition in a subject comprising administering to the subject an effective amount of an agent, wherein the agent regulates the PEMT activity.

100. A method for treating or preventing a neurological condition in a subject comprising administering to the maternal host of the subject an effective amount of an agent, wherein the agent regulates the PEMT activity of the maternal host of the subject.

101. The method of claim 99, wherein the PEMT activity is in liver, intestine, brain, or mammary gland.

102. The method of claim 100, wherein the PEMT activity is in liver or mammary gland.
103. The method of claim 100, wherein the subject is a fetus or an infant.

104. The method of claim 100, wherein the subject is an infant and intakes milk from the maternal host.

105. The method of claim 99, wherein the agent increases the PEMT activity.

106. The method of claim 99, wherein the agent increases the PEMT activity and is selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-1 adrenergic receptor agonist, β-2 adrenergic receptor agonist, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

107. The method of claim 100, wherein the agent increases the PEMT activity.

108. The method of claim 100, wherein the agent increases the PEMT activity and is selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-1 adrenergic receptor agonist, β-2 adrenergic receptor agonist, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

109. The method of claim 99, wherein the neurological condition is neurodevelopment or neurodegeneration of the subject.

110. The method of claim 99, wherein the neurological condition is selected from the group consisting of Asperger’s Syndrome, Attention Deficit Hyperactivity Disorder, Autism spectrum disorder, Cerebral Palsy, Dysthymic Disorder, Fragile X Syndrome, Perinatally Acquired HIV Disease, Tourette’s Syndrome, Alternating Hemiplegia of Childhood, Congenital Anomalies, Arnold-Chiari Malformation,
Meningocele, Spina Bifida, Dandy Walker Syndrome, vascular malformations, Holoprosencephaly, and Hydranencephaly.

111. The method of claim 100, wherein the neurological condition is neurodevelopment of the subject.

112. The method of claim 100, wherein the neurological condition is selected from the group consisting of Asperger's Syndrome, Attention Deficit Hyperactivity Disorder, Autism spectrum disorder, Cerebral Palsy, Dysthymic Disorder, Fragile X Syndrome, Perinatally Acquired HIV Disease, Tourette’s Syndrome, Alternating Hemiplegia of Childhood, Congenital Anomalies, Arnold-Chiari Malformation, Meningocele, Spina Bifida, Dandy Walker Syndrome, vascular malformations, Holoprosencephaly, and Hydranencephaly.

113. The method of claim 99, wherein the neurological condition is selected from the group consisting of Alpers’ disease, Alzheimer's Disease, Autosomal Dominant Neurodegenerative Disorder, Batten Disease, Cerebral calcnosis, Cockayne Syndrome, corticobasal ganglionic degeneration, Dementia with Lewy Bodies, Lewy Body Variant, Alzheimers Disease, Motor Neuron Disease, Multiple System Atrophy, Parkinson Plus syndrome, Neuronal intranuclear inclusion disease, Olivopontocerebellar Atrophy, Parkinsonian Syndromes, Pick's disease, Postpoliomyelitis Syndrome, Progressive Supranuclear Palsy, Rett Syndrome, Shy-Drager Syndrome, Tauopathies, Tri-nucleotide repeat diseases, and Tuberous Sclerosis.

114. The method of claim 99, wherein the neurological condition is neurodevelopment of the subject exposed to alcohol consumption.

115. The method of claim 99, wherein the neurological condition is fetal alcohol syndrome.
116. The method of claim 100, wherein the neurological condition is fetal alcohol syndrome.

117. A method for treating or preventing a neurological condition in a subject comprising administering to the subject an effective amount of an agent selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-1 adrenergic receptor agonist, β-2 adrenergic receptor agonist, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

118. A method for treating or preventing a neurological condition in a subject comprising administering to the maternal host of the subject an effective amount of an agent selected from the group consisting of folic acid, vitamin B6, growth hormone, sex hormone, β-1 adrenergic receptor agonist, β-2 adrenergic receptor agonist, β-3 adrenergic receptor agonist, niacin, and S-adenosylmethionine.

119. The method of claim 117, wherein the neurological condition is neurodevelopment or neurodegeneration of the subject.

120. The method of claim 117, wherein the neurological condition is selected from the group consisting of Asperger’s Syndrome, Attention Deficit Hyperactivity Disorder, Autism spectrum disorder, Cerebral Palsy, Dysthyemic Disorder, Fragile X Syndrome, Perinatally Acquired HIV Disease, Tourette’s Syndrome, Alternating Hemiplegia of Childhood, Congenital Anomalies, Arnold-Chiari Malformation, Meningocele, Spina Bifida, Dandy Walker Syndrome, vascular malformations, Holoprosencephaly, and Hydranencephaly.

121. The method of claim 117, wherein the neurological condition is selected from the group consisting of Alpers’ disease, Alzheimer's Disease, Autosomal Dominant Neurodegenerative Disorder, Batten Disease, Cerebral calcinos, Cockayne Syndrome, corticobasal

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122. The method of claim 117, wherein the neurological condition is neurodevelopment of the subject exposed to alcohol consumption.

123. The method of claim 117, wherein the neurological condition is fetal alcohol syndrome.

124. A method for assessing the susceptibility of a subject to the toxicity of an agent comprising determining the level of PEMT activity in the subject, wherein a level of PEMT activity that is lower than a normal PEMT activity is indicative of the susceptibility of the subject to the toxicity of the agent.

125. The method of claim 124, wherein the agent is a therapeutic agent.

126. The method of claim 124, wherein the agent is from an environment.

127. The method of claim 124, wherein the subject is human.

128. A method for assessing the susceptibility of a subject to the toxicity of an agent comprising determining the level of CDP-choline activity in the subject, wherein a level of CDP-choline activity that is lower than a normal CDP-choline activity is indicative of the susceptibility of the subject to the toxicity of the agent.
129. The method of claim 128, wherein the agent is a therapeutic agent.

130. The method of claim 128, wherein the agent is from an environment.

131. The method of claim 128, wherein the subject is human.

132. The method of claim 128, wherein the level of CDP-choline activity is in plasma.

133. A method for assessing the susceptibility of a subject to a condition associated with infertility comprising determining the level of PEMT activity in the subject, wherein a lower than normal PEMT activity is indicative of the susceptibility of the subject to a condition associated with infertility.

134. The method of claim 133, wherein the condition associated with infertility is lower than normal sperm motility.

135. A method for assessing the exposure of a subject to an agent in an environment comprising determining the level of PEMT activity in the subject, wherein a lower than normal PEMT activity is indicative of a higher than normal exposure of the subject to the agent in the environment and wherein the agent is associated with infertility or gender disorder.

136. The method of claim 135, wherein the agent is dioxins or an anti-estrogen agent.

137. A method for assessing a condition in a subject comprising determining the level of PEMT activity in a subject, wherein the level of PEMT activity corresponds to the level of a condition selected from the
group consisting of growth hormone, thyroid activity, eicosanoid production, insulin, and insulin resistance in the subject.

138. A method for predicting the effectiveness of an agent for the treatment of a neoplasia comprising determining the level of PEMT activity in a subject in response to the agent, wherein a decrease in the PEMT activity in response to the agent is predictive of the effectiveness of the agent for the treatment of the neoplasia.

139. The method of claim 138, wherein the agent is PPAR agonist or nuclear hormone receptor agonist.

140. The method of claim 138, wherein the neoplasia is responsive to PPAR and is selected from the group consisting of colon cancer, thyroid cancer, pancreatic cancer, esophageal squamous carcinoma, and prostate cancer.

141. A method of increasing milk production in a subject comprising administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases milk production of the subject.

142. A method of increasing the lipid content of milk in a subject comprising administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases the lipid content of milk, wherein the lipid is 22:6n3 or 20:4n6.

143. A method of increasing the phospholipid content of milk in a subject comprising administering to a subject in need of such treatment an agent, wherein the agent increases PEMT activity and whereby increases the phospholipid content of milk.

144. A method of assessing the susceptibility of a subject to a developmental condition comprising determining the level of PEMT
activity in the subject, wherein a lower than normal level of PEMT activity in the subject is indicative of susceptibility of the subject to a developmental condition selected from the group consisting of birth defects, congenital developmental conditions, Microcephaly and mental retardation of uncontrolled phenylketonuria (PKU).

145. A method of assessing the susceptibility of a subject to a developmental condition comprising determining the level of PEMT activity in the maternal host of the subject, wherein a lower than normal level of PEMT activity in the maternal host of the subject is indicative of susceptibility of the subject to a developmental condition selected from the group consisting of birth defects, congenital developmental conditions, Microcephaly and mental retardation of uncontrolled phenylketonuria (PKU).

146. The method of claim 145, wherein the level of PEMT activity in the maternal host of the subject is determined prior to the maternal host giving birth to the subject.

147. The method of claim 145, wherein the level of PEMT activity in the maternal host of the subject is determined during a period when the subject intakes milk from the maternal host.

148. A method of preventing a birth defect of a subject comprising administering to the maternal host of the subject an agent, wherein the agent increases PEMT activity, 22:6n3, or 20:4n6 in the maternal host of the subject.

149. The method of claim 148, wherein the PEMT activity is in liver or mammary gland of the maternal host of the subject.

150. The method of claim 148, wherein the birth defect is associated with atopic diseases, diabetes, immune function, psychomotor development, visual and stereo acuity, and action of probiotics.
151. The method of claim 148, wherein the subject intakes milk from the maternal host.

152. A method for preventing or treating a birth defect of a subject comprising administering to the subject an agent, wherein the agent increases the level of PEMT activity, 22:6n3, or 20:4n6 in the subject.

153. The method of claim 152, wherein the agent is an intake supplement for the subject and is selected from the group consisting of polyunsaturated fatty acid, 22:6n3, and 20:4n6.

154. A method of treating or preventing a condition associated with the PEMT activity in a subject comprising administering to the subject an effective amount of an agent, wherein the agent regulates PEMT activity and wherein the condition associated with the PEMT activity is selected from the group consisting of cirrhosis, skin disorders, neoplasia, infectious diseases, inflammatory diseases, bone density, eicosanoid production, osteoporosis, insulin sensitivity or resistance, diabetes, obesity, renal function loss, autoimmune diseases, and atopic diseases, lipid accumulation in liver, liver steatosis, complications associated with liver transplantation, growth hormone deficiency or overproduction, and thyroid hormone disorders.

155. The method of claim 154, wherein the condition is selected from the group consisting of skin disorders, infectious diseases, rheumatoid arthritis, osteoporosis, atopic diseases, cirrhosis, renal functional loss, autoimmune diseases, lipid accumulation in liver, complications associated with liver transplantation, insulin resistance, growth hormone deficiency or excess, and thyroid hormone disorders, and the agent increases PEMT activity.
156. The method of claim 154, wherein the condition is selected from the group consisting of insulin resistance, neoplasia, chronic bowel inflammation, and the agent decreases PEMT activity.
Figure 2M

Figure 2N