BIOMARKERS FOR PRE-DIABETES, CARDIOVASCULAR DISEASES, AND OTHER METABOLIC-SYNDROME RELATED DISORDERS AND METHODS USING THE SAME

Cross-Reference to Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 60/950,286, filed July 17, 2007, and U.S. Provisional Application No. 61/037,628, filed March 18, 2008, the entireties of which are hereby incorporated by reference herein.

Field

[0002] The invention generally relates to biomarkers, methods for identifying biomarkers correlated to insulin resistance, cardiovascular disease, and metabolic-syndrome-related disorders and methods based on the same biomarkers.

Background

[0003] Diabetes is classified as either type 1 (early onset) or type 2 (adult onset), with type 2 comprising 90-95% of the cases of diabetes. Diabetes is the final stage in a disease process that begins to affect individuals long before the diagnosis of diabetes is made. Type 2 diabetes develops over 10 to 20 years and results from an impaired ability to utilize glucose (glucose utilization) due to impaired sensitivity to insulin (insulin resistance).

[0004] In pre-diabetes, insulin becomes less effective at helping tissues metabolize glucose. Pre-diabetics may be detectable as early as 20 years before diabetic symptoms become evident. Studies have shown that although patients show very few symptoms, long-term physiological damage is already occurring at this stage. Up to 60% of these individuals will progress to type 2 diabetes within 10 years.

[0005] The American Diabetes Association (ADA) has recommended routine screening to detect patients with pre-diabetes. Current screening methods for pre-diabetes include the fasting plasma glucose (FPG) test, the oral glucose tolerance test (OGTT), the fasting insulin test and the hyperinsulinemic euglycemic clamp (HI clamp).

The first two tests are used clinically whereas the latter two tests are used extensively in research but rarely in the clinic. In addition, mathematical means (e.g., HOMA, QUICKI) that consider the fasting glucose and insulin levels together have been proposed. However, normal plasma insulin concentrations vary considerably between individuals as well as within an individual throughout the day. Further, these methods suffer from variability and methodological differences between laboratories and do not correlate rigorously with glucose clamp studies.

[0006] Worldwide, an estimated 194 million adults have type 2 diabetes and this number is expected to increase to 333 million by 2025, largely due to the epidemic of obesity in westernized societies. In the United States, it is estimated that over 54 million adults are pre-diabetic, depending on the level of insulin resistance. There are approximately 1.5 million new cases of type 2 diabetes a year in the United States. The annual US healthcare cost for diabetes is estimated at $174 billion. This figure has risen more than 32% since 2002. In industrialized countries such as the U.S., about 25% of medical expenditures treat glycemic control, 50% is associated with general medical care associated with diabetes, and the remaining 25% of the costs go to treat long-term complications, primarily cardiovascular disease. Considering the distribution of the healthcare costs and the fact that insulin resistance is a direct causal factor in cardiovascular disease and diabetes progression, it is no surprise that cardiovascular disease accounts for 70-80% of the mortality observed for diabetic patients. Detecting and preventing type 2 diabetes has become a major health care priority.

[0007] Diabetes may also lead to the development of other diseases or conditions, or is a risk factor in the development of conditions such as Metabolic Syndrome and cardiovascular diseases. Metabolic Syndrome is the clustering of a set of risk factors in an individual. According to the American Heart Association these risk factors include: abdominal obesity, decreased ability to properly process glucose (insulin resistance or glucose intolerance), dyslipidemia (high triglycerides, high LDL, low HDL cholesterol), hypertension, prothrombotic state (high fibrinogen or plasminogen activator inhibitor-1 in the blood) and proinflammatory state (elevated C-reactive protein in the blood). Metabolic Syndrome is also known as syndrome X, insulin resistance syndrome, obesity syndrome, dysmetabolic syndrome and Reaven's syndrome. Patients diagnosed with

Metabolic Syndrome are at an increased risk of developing diabetes, cardiac and vascular disease. It is estimated that, in the United States, 20% of the adults (>50 million people) have metabolic syndrome. While it can affect anyone at any age, the incidence increases with increasing age and in individuals who are inactive, and significantly overweight, especially with excess abdominal fat.

[0008] Type 2 diabetes is the most common form of diabetes in the United States. According to the American Diabetes Foundation over 90% of the US diabetics suffer from Type 2 diabetes. Individuals with Type 2 diabetes have a combination of increased insulin resistance and decreased insulin secretion that combine to cause hyperglycemia. Most persons with Type 2 diabetes have Metabolic Syndrome.

[0009] The diagnosis for Metabolic Syndrome is based upon the clustering of three or more of the risk factors in an individual. There are no well-accepted criteria for diagnosing the metabolic syndrome. The criteria proposed by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III), with minor modifications, are currently recommended and widely used.

[0010] The American Heart Association and the National Heart, Lung, and Blood Institute recommend that the metabolic syndrome be identified as the presence of three or more of these components: increased waist circumference (Men—equal to or greater than 40 inches (102 cm), Women—equal to or greater than 35 inches (88 cm); elevated triglycerides (equal to or greater than 150 mg/dL); reduced HDL ("good") cholesterol (Men—less than 40 mg/dL, Women—less than 50 mg/dL); elevated blood pressure (equal to or greater than 130/85 mm Hg); elevated fasting glucose (equal to or greater than 100 mg/dL).

[0011] Type 2 diabetes develops slowly and often people first learn they have type 2 diabetes through blood tests done for another condition or as part of a routine exam. In some cases, type 2 diabetes may not be detected before damage to eyes, kidneys or other organs has occurred. A need exists for an objective, biochemical evaluation (e.g. lab test) that can be administered by a primary care provider to identify individuals that are at risk of developing Metabolic Syndrome or Type 2 diabetes.

[0012] Newer, more innovative molecular diagnostics that reflect the mechanisms of the patho-physiological progression to pre-diabetes and diabetes are needed because the

prevalence of pre-diabetes and diabetes is increasing in global epidemic proportions. Mirroring the obesity epidemic, pre-diabetes and diabetes are largely preventable but are frequently undiagnosed or diagnosed too late due to the asymptomatic nature of the progression to clinical disease.

[0013] Therefore there is an unmet need for diagnostic biomarkers and tests that can identify pre-diabetics at risk of developing type 2 diabetes and to determine the risk of disease progression in subjects with insulin resistance. Insulin resistance biomarkers and diagnostic tests can better identify and determine the risk of diabetes development in a pre-diabetic subject, can monitor disease development and progression and/or regression, can allow new therapeutic treatments to be developed and can be used to test therapeutic agents for efficacy on reversing pre-diabetes and/or preventing diabetes. Further, a need exists for diagnostic biomarkers to more effectively assess the efficacy and safety of pre-diabetic and diabetic therapeutic candidates.

Summary of the Invention

[0014] In one embodiment, the present disclosure provides a method of diagnosing insulin resistance in a subject, the method comprising analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for insulin resistance in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, 29 and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to insulin resistance-positive and/or insulin resistance-negative reference levels of the one or more biomarkers in order to diagnose whether the subject is insulin resistant.

[0015] In another embodiment, the present disclosure provides a method of predicting the glucose disposal rate in a subject, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for insulin resistance in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to glucose disposal reference levels of the one or more biomarkers in order to predict the glucosal disposal rate in the subject.

[0016] The disclosure also provides a method of classifying a subject according to

glucose tolerance from normal glucose tolerance (NGT), impaired fasting glucose tolerance (IFG), or impaired glucose tolerance (IGT), to type-2 diabetes, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for glucose tolerance in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to glucose tolerance reference levels of the one or more biomarkers in order to classify the subject as having NGT, IFG, IGT, or diabetic.

[0017] Further provided is a method of determining susceptibility of a subject to developing type-2 diabetes, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to diabetes-positive and/or diabetes-negative reference levels of the one or more biomarkers in order to diagnose whether the subject is susceptible to developing type-2 diabetes.

[0018] The present disclosure also provides a method of determining an insulin resistance score in a subject, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to insulin resistance reference levels of the one or more biomarkers in order to determine an insulin resistance score for the subject.

[0019] In another embodiment, the present disclosure provides a method of monitoring the progression or regression of pre-diabetes in a subject, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to pre-diabetes progression and/or pre-diabetes-regression reference levels of the one or more biomarkers in order to monitor the progression or regression of pre-diabetes

in a subject.

[0020] In yet another embodiment, the present disclosure provides a method of monitoring the efficacy of insulin resistance treatment, the method comprising: analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, the first sample obtained from the subject at a first time point wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, and 8, and combinations thereof; treating the subject for insulin resistance; analyzing a second biological sample from the subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point after treatment; comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample to assess the efficacy of the treatment for treating insulin resistance.

[0021] The present disclosure further provides a method of diagnosing whether a subject has metabolic syndrome, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic syndrome in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 12 and 13, analyzing the biological sample to determine the level(s) of one or more biomarkers for glucose disposal, obesity, and/or cardiovascular disease, wherein the one or more biomarkers for glucose disposal, obesity, and/or cardiovascular disease are selected from one or more biomarkers identified in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to the respective disorder-positive and/or disorder-negative reference levels of the one or more biomarkers in order to diagnose whether the subject has the metabolic syndrome.

[0022] In another embodiment, the present disclosure provides a method of diagnosing a cardiovascular disease in a subject, the method comprising, analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for a cardiovascular disease in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Tables 14, 15, 16, 17, 21, 22, 23, 25, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to disease-positive and/or disease-negative reference levels of the one or more

biomarkers in order to diagnose whether the subject has cardiomyopathy or atherosclerosis.

[0023] The disclosure further provides a method for determining whether a subject is predisposed to becoming obese, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for obesity in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Table 26; and comparing the level(s) of the one or more biomarkers in the sample to obesity-positive and/or obesity-negative and/or lean-positive and/or lean-negative reference levels of the one or more biomarkers in order to determine whether the subject is susceptible to obesity.

[0024] In yet a further embodiment, the disclosure provides a method for determining whether a therapeutic agent is capable of inducing weight gain in a subject, the method comprising: analyzing a biological sample from a subject receiving a therapeutic agent to determine the level(s) of one or more biomarkers for obesity in the sample, where the one or more biomarkers are selected from one or more biomarkers listed in Table 26; and comparing the level(s) of the one or more biomarkers in the sample to obesity-positive and/or obesity-negative and/or lean-positive and/or lean-negative reference levels of the one or more biomarkers in order to determine whether the subject is susceptible to gaining weight.

[0025] The present disclosure also provides a method for predicting a subject's response to a course of treatment for pre-diabetes or diabetes, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, where the one or more biomarkers are selected from one or more biomarkers listed in Table 27; comparing the level(s) of one or more biomarkers in the sample to treatment-positive and/or treatment-negative reference levels of the one or more biomarkers to predict whether the subject is likely to respond to a course of treatment.

[0026] The disclosure also provides a method for monitoring a subject's response to a treatment for pre-diabetes or diabetes, the method comprising: analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, the first sample obtained from the subject at a first time point where the one or

more biomarkers are selected from one or more biomarkers listed in Table 28; administering the composition to the subject; analyzing a second biological sample from the subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point after administration of the composition; comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample to assess the efficacy of the composition for treating pre-diabetes or diabetes.

Brief Description of the Drawings

[0027] Figure 1 provides a graph showing the mean R^2 values of Rd correlation as a function of the number of metabolites. As the number of compounds increases (from right to left), the r-square value for Rd correlation (Y) increases until it reaches an optimal number ($n<30$), indicating that variable selection is more or less stable for the approximately first 30 variables.

[0028] Figure 2 provides a graph showing the mean R^2 values of Rd correlation as a function of the number of metabolites. As the number of compounds increases (from right to left), test error for Rd correlation (Y) decreases until it reach an optimal number ($n<30$), indicating that variable selection is more or less stable for the approximately first 30 variables.

[0029] Figure 3 provides a graph showing the mean R-square values (Y-axis) of Rd correlation as a function of the number of metabolites (X-axis).

[0030] Figure 4 provides a graph showing the mean test error values (Y-axis) of Rd correlation as a function of the number of metabolites (X-axis).

[0031] Figure 5 provides a schematic example of a report describing prophetic results of an algorithm using insulin resistance biomarkers to determine a subject's level of insulin resistance that is reported as an "IR Score" and containing additional clinical information (e.g. BMI, demographic information).

[0032] Figure 6 provides a schematic illustration comparing the use of biomarkers for patient stratification according to the level of insulin resistance and the use of biomarkers for patient risk stratification for the progression of insulin resistance.

[0033] Figure 7 provides a Random Forest Analysis Importance plot of one embodiment of metabolites that are useful biomarkers for predicting glucose disposal.

[0034] Figure 8 provides a Random Forest Analysis Importance Plot of one embodiment of serum metabolites that are useful biomarkers for predicting metabolic syndrome.

[0035] Figure 9 provides a Random Forest Analysis Importance Plot of one embodiment of plasma metabolites that are useful biomarkers for predicting metabolic syndrome.

[0036] Figure 10 provides a Random Forest Analysis Importance Plots of embodiments of metabolites from plasma that are useful biomarkers for predicting atherosclerosis at early (initiation) (Figure 10A), mid (Figure 10B), later (Figure 10C), or all (Figure 10D) stages of the disease.

[0037] Figure 11 provides a Random Forest Analysis Importance Plots of embodiments of metabolites from aorta tissue that are useful biomarkers for predicting atherosclerosis at early (initiation) (Figure 11A), mid (Figure 11B), later (Figure 11C), or all (Figure 11D) stages of the disease.

[0038] Figure 12 provides a Random Forest Analysis Importance Plots of embodiments of metabolites from liver tissue that are useful biomarkers for predicting atherosclerosis at early (initiation) (Figure 12A), mid (Figure 12B), later (Figure 12C), or all (Figure 12D) stages of the disease.

[0039] Figure 13 provides an example of plasma levels of cholesterol in atherosclerosis subjects and control subjects at different ages.

[0040] Figure 14 provides an example of plasma levels of docosahexaenoic acid in atherosclerosis subjects and control subjects at different ages.

[0041] Figure 15 provides an example of plasma levels of Metabolite-7888 in atherosclerosis subjects and control subjects at different ages.

[0042] Figure 16 provides an example of plasma levels of Metabolite-X8403 in atherosclerosis subjects and control subjects at different ages.

[0043] Figure 17 provides an example of plasma levels of Metabolite-X1834 in atherosclerosis subjects and control subjects at different ages.

[0044] Figure 18 provides an example of plasma levels of p-cresol-sulfate in atherosclerosis subjects and control subjects at different ages.

[0045] Figure 19 provides an example of plasma levels of Metabolite-4887 in

atherosclerosis subjects and control subjects at different ages.

[0046] Figure 20 provides an example of recursive partitioning of DCM biomarker metabolites.

[0047] Figure 21 provides an example of model validation using plasma from metabolic syndrome and healthy subjects.

[0048] Figure 22 provides an example of a model validation using serum from metabolic syndrome and healthy subjects.

[0049] Figure 23 provides an example of a regression analysis showing the predictive power of the ten models combined on the glucose disposal rate (Rd).

[0050] Figure 24 provides and illustration of the inter-relationships of the various risk factors for metabolic syndrome.

Detailed Description of the Invention

[0051] The present invention relates to biomarkers of pre-diabetes (e.g. impaired glucose tolerance, impaired fasting glucose tolerance, insulin resistance) and type-2 diabetes; methods for diagnosis of pre-diabetes and type 2 diabetes; methods of determining predisposition to pre-diabetes and type 2 diabetes; methods of monitoring progression/regression of pre-diabetes and type 2 diabetes; methods of assessing efficacy of compositions for treating pre-diabetes and type 2 diabetes; methods of screening compositions for activity in modulating biomarkers of pre-diabetes and type 2 diabetes; methods of treating pre-diabetes and type 2 diabetes; as well as other methods based on biomarkers of pre-diabetes and type 2 diabetes.

[0052] Current blood tests for insulin resistance perform poorly for early detection of insulin resistance or involve significant medical procedures.

[0053] Using metabolomic analysis, panels of metabolites that can be used in a simple blood test to predict insulin resistance as measured by the "gold standard" of hyperinsulinemic euglycemic clamps in at least two independent cohorts of subjects were discovered.

[0054] Independent studies were carried out to identify a set of biomarkers that when used with a polynomic algorithm will enable the early detection of changes in insulin resistance in a subject. The instant invention provides the subject with a score indicating

the level of insulin resistance ("IR Score") of the subject. The score can be based upon clinically significant changed reference level for a biomarker and/or combination of biomarkers. The reference level can be derived from an algorithm or computed from indices for impaired glucose tolerance and can be presented in a report as shown in Figure 5. The IR Score places the subject in the range of insulin resistance from normal to high. Disease progression or remission can be monitored by periodic determination and monitoring of the IR Score. Response to therapeutic intervention can be determined by monitoring the IR Score. The IR Score can also be used to evaluate drug efficacy.

[0055] The present invention also relates to biomarkers of metabolic syndrome and cardiovascular diseases, such as atherosclerosis and cardiomyopathy; methods for diagnosis of such diseases and syndromes; methods of determining predisposition to such diseases and syndromes; methods of monitoring progression/regression of such diseases and syndromes; methods of assessing efficacy of compositions for treating such diseases and syndromes; methods of screening compositions for activity in modulating biomarkers of such diseases and syndromes; methods of treating such diseases and syndromes; as well as other methods based on biomarkers of such diseases and syndromes.

[0056] Prior to describing this invention in further detail, however, the following terms will first be defined.

Definitions:

[0057] "Biomarker" means a compound, preferably a metabolite, that is differentially present (i.e., increased or decreased) in a biological sample from a subject or a group of subjects having a first phenotype (e.g., having a disease) as compared to a biological sample from a subject or group of subjects having a second phenotype (e.g., not having the disease). A biomarker may be differentially present at any level, but is generally present at a level that is increased by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 100%, by at least 110%, by at least 120%, by at least 130%, by at least 140%, by at least 150%, or more; or is generally present at a level that is decreased by at least 5%, by

at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, or by 100% (i.e., absent). A biomarker is preferably differentially present at a level that is statistically significant (e.g., a p-value less than 0.05 and/or a q-value of less than 0.10 as determined using either Welch's T-test or Wilcoxon's rank-sum Test). Alternatively, the biomarkers demonstrate a correlation with pre-diabetes, or particular levels of pre-diabetes. The range of possible correlations is between negative (-) 1 and positive (+) 1. A result of negative (-) 1 means a perfect negative correlation and a positive (+) 1 means a perfect positive correlation, and 0 means no correlation at all. A "substantial positive correlation" refers to a biomarker having a correlation from +0.25 to +1.0 with a disorder or with a clinical measurement (e.g., Rd), while a "substantial negative correlation" refers to a correlation from -0.25 to -1.0 with a given disorder or clinical measurement. A "significant positive correlation" refers to a biomarker having a correlation of from +0.5 to +1.0 with a given disorder or clinical measurement (e.g., Rd), while a "significant negative correlation" refers to a correlation to a disorder of from -0.5 to -1.0 with a given disorder or clinical measurement.

[0058] The "level" of one or more biomarkers means the absolute or relative amount or concentration of the biomarker in the sample.

[0059] "Sample" or "biological sample" or "specimen" means biological material isolated from a subject. The biological sample may contain any biological material suitable for detecting the desired biomarkers, and may comprise cellular and/or non-cellular material from the subject. The sample can be isolated from any suitable biological tissue or fluid such as, for example, adipose tissue, aortic tissue, liver tissue, blood, blood plasma, serum, or urine.

[0060] "Subject" means any animal, but is preferably a mammal, such as, for example, a human, monkey, non-human primate, rat, mouse, cow, dog, cat, pig, horse, or rabbit.

[0061] A "reference level" of a biomarker means a level of the biomarker that is indicative of a particular disease state, phenotype, or lack thereof, as well as

combinations of disease states, phenotypes, or lack thereof. A "positive" reference level of a biomarker means a level that is indicative of a particular disease state or phenotype. A "negative" reference level of a biomarker means a level that is indicative of a lack of a particular disease state or phenotype. For example, a "pre-diabetes-positive reference level" of a biomarker means a level of a biomarker that is indicative of a positive diagnosis of pre-diabetes in a subject, and a "pre-diabetes-negative reference level" of a biomarker means a level of a biomarker that is indicative of a negative diagnosis of pre-diabetes in a subject. As another example, a "pre-diabetes-progression-positive reference level" of a biomarker means a level of a biomarker that is indicative of progression of the pre-diabetes in a subject, and a "pre-diabetes-regression-positive reference level" of a biomarker means a level of a biomarker that is indicative of regression of the pre-diabetes. A "reference level" of a biomarker may be an absolute or relative amount or concentration of the biomarker, a presence or absence of the biomarker, a range of amount or concentration of the biomarker, a minimum and/or maximum amount or concentration of the biomarker, a mean amount or concentration of the biomarker, and/or a median amount or concentration of the biomarker; and, in addition, "reference levels" of combinations of biomarkers may also be ratios of absolute or relative amounts or concentrations of two or more biomarkers with respect to each other. Appropriate positive and negative reference levels of biomarkers for a particular disease state, phenotype, or lack thereof may be determined by measuring levels of desired biomarkers in one or more appropriate subjects, and such reference levels may be tailored to specific populations of subjects (e.g., a reference level may be age-matched so that comparisons may be made between biomarker levels in samples from subjects of a certain age and reference levels for a particular disease state, phenotype, or lack thereof in a certain age group). Such reference levels may also be tailored to specific techniques that are used to measure levels of biomarkers in biological samples (e.g., LC-MS, GC-MS, etc.), where the levels of biomarkers may differ based on the specific technique that is used.

[0062] "Non-biomarker compound" means a compound that is not differentially present in a biological sample from a subject or a group of subjects having a first phenotype (e.g., having a first disease) as compared to a biological sample from a subject or group of subjects having a second phenotype (e.g., not having the first disease). Such

non-biomarker compounds may, however, be biomarkers in a biological sample from a subject or a group of subjects having a third phenotype (e.g., having a second disease) as compared to the first phenotype (e.g., having the first disease) or the second phenotype (e.g., not having the first disease).

[0063] "Metabolite", or "small molecule", means organic and inorganic molecules which are present in a cell. The term does not include large macromolecules, such as large proteins (e.g., proteins with molecular weights over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000), large nucleic acids (e.g., nucleic acids with molecular weights of over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000), or large polysaccharides (e.g., polysaccharides with a molecular weights of over 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, or 10,000). The small molecules of the cell are generally found free in solution in the cytoplasm or in other organelles, such as the mitochondria, where they form a pool of intermediates which can be metabolized further or used to generate large molecules, called macromolecules. The term "small molecules" includes signaling molecules and intermediates in the chemical reactions that transform energy derived from food into usable forms. Examples of small molecules include sugars, fatty acids, amino acids, nucleotides, intermediates formed during cellular processes, and other small molecules found within the cell.

[0064] "Metabolic profile", or "small molecule profile", means a complete or partial inventory of small molecules within a targeted cell, tissue, organ, organism, or fraction thereof (e.g., cellular compartment). The inventory may include the quantity and/or type of small molecules present. The "small molecule profile" may be determined using a single technique or multiple different techniques.

[0065] "Metabolome" means all of the small molecules present in a given organism.

[0066] "Metabolic disorder" refers to disorders or diseases that result in perturbation of the normal physiological state of homeostasis due to an alteration in metabolism (anabolism and/or catabolism). An alteration in metabolism can result from an inability to break down (catabolize) a substance that should be broken down (*e.g.* phenylalanine) and as a result the substance and/or an intermediate substance builds up to toxic levels, or from an inability to produce (anabolize) some essential substance (*e.g.* insulin).

[0067] "Metabolic syndrome" refers to the concept of a clustering of metabolic risk

factors that come together in a single individual and lead to a high risk of developing diabetes and/or cardiovascular diseases. The main features of metabolic syndrome include insulin resistance, hypertension (high blood pressure), cholesterol abnormalities, dyslipidemia, triglyceride abnormalities, an increased risk for clotting and excess body weight, especially in the abdomen, or obesity. Metabolic syndrome is also known as syndrome X, insulin resistance syndrome, obesity syndrome, dysmetabolic syndrome and Reaven's syndrome. The inter-relationships of the various risk factors for metabolic syndrome are illustrated in Figure 24. The presence of three or more of the risk factors in a single individual is indicative of metabolic syndrome. The American Heart Association suggests that metabolic syndrome be diagnosed by the presence of three or more of the following components: (1) an elevated waste circumference (men, equal to or greater than 40 inches (102cm); women, equal to or greater than 35 inches (88cm)); (2) elevated triglycerides (equal to or greater than 150 mg/dL); (3) reduced High Density Lipids or HDL (men, less than 40 mg/dL; women, less than 50 mg/dL); (4) elevated blood pressure (equal to or greater than 130/85 mm Hg); and (5) elevated fasting glucose (equal to or greater than 100 mg/dL).

[0068] "Metabolic syndrome-related metabolic disorder" as used herein refers to metabolic syndrome as well as obesity, insulin resistance, type-2 diabetes, atherosclerosis, and cardiomyopathy.

[0069] "Diabetes" refers to a group of metabolic diseases characterized by high blood sugar (glucose) levels which result from defects in insulin secretion or action, or both.

[0070] "Type 2 diabetes" refers to one of the two major types of diabetes, the type in which the beta cells of the pancreas produce insulin, at least in the early stages of the disease, but the body is unable to use it effectively because the cells of the body are resistant to the action of insulin. In later stages of the disease the beta cells may stop producing insulin. Type 2 diabetes is also known as insulin-resistant diabetes, non-insulin dependent diabetes and adult-onset diabetes.

[0071] "Pre-diabetes" refers to one or more early diabetic conditions including impaired glucose utilization, abnormal or impaired fasting glucose levels, impaired glucose tolerance, impaired insulin sensitivity and insulin resistance.

[0072] "Insulin resistance" refers to the condition when cells become resistant to the

effects of insulin — a hormone that regulates the uptake of glucose into cells — or when the amount of insulin produced is insufficient to maintain a normal glucose level. Cells are diminished in the ability to respond to the action of insulin in promoting the transport of the sugar glucose from blood into muscles and other tissues(i.e. sensitivity to insulin decreases). Eventually, the pancreas produces far more insulin than normal and the cells continue to be resistant. As long as enough insulin is produced to overcome this resistance, blood glucose levels remain normal. Once the pancreas is no longer able to keep up, blood glucose starts to rise, resulting in diabetes. Insulin resistance ranges from normal (insulin sensitive) to insulin resistant (IR).

[0073] "Insulin sensitivity" refers to the ability of cells to respond to the effects of insulin to regulate the uptake and utilization of glucose. Insulin sensitivity ranges from normal to Insulin Resistant (IR).

[0074] The "IR Score" is a measure of insulin resistance based upon the insulin resistance biomarkers and algorithms of the instant invention that will allow the physician to place the patient on the spectrum of glucose tolerance, from normal to high.

[0075] "Glucose utilization" refers to the absorption of glucose from the blood by muscle and fat cells and utilization of the sugar for cellular metabolism. The uptake of glucose into cells is stimulated by insulin.

[0076] "Rd" refers to glucose disposal rate, a metric for glucose utilization. The rate at which glucose disappears from the blood (disposal rate) is an indication of the ability of the body to respond to insulin (i.e. insulin sensitivity). There are several methods to determine Rd and the hyperinsulinemic euglycemic clamp is regarded as the "gold standard" method. In this technique, while a fixed amount of insulin is infused, the blood glucose is "clamped" at a predetermined level by the titration of a variable rate of glucose infusion. The underlying principle is that upon reaching steady state, by definition, glucose disposal is equivalent to glucose appearance. During hyperinsulinemia, glucose disposal (Rd) is primarily accounted for by glucose uptake into skeletal muscle, and glucose appearance is equal to the sum of the exogenous glucose infusion rate plus the rate of hepatic glucose output (HGO). The rate of glucose infusion during the last 30 minutes of the test determines insulin sensitivity. If high levels (Rd = 7.5 mg/min or higher) are required, the patient is insulin-sensitive. Very low levels (Rd = 4.0 mg/min or

lower) indicate that the body is resistant to insulin action. Levels between 4.0 and 7.5 mg/min (Rd values between 4.0 mg/min and 7.5 mg/min) are not definitive and suggest "impaired glucose tolerance," an early sign of insulin resistance.

[0077] "Impaired fasting glucose (IFG)" and "impaired glucose tolerance (IGT)" are the two clinical definitions of "pre-diabetes". IFG is defined as a fasting blood glucose concentration of 100-125 mg/dL. IGT is defined as a postprandial (after eating) blood glucose concentration of 140-199 mg/dL. It is known that IFG and IGT do not always detect the same pre-diabetic populations. Between the two populations there is approximately a 60% overlap observed. Fasting plasma glucose levels are a more efficient means of inferring a patient's pancreatic function, or insulin secretion, whereas postprandial glucose levels are more frequently associated with inferring levels of insulin sensitivity or resistance. IGT is known to identify a greater percentage of the pre-diabetic population compared to IFG. The IFG condition is associated with lower insulin secretion, whereas the IGT condition is known to be strongly associated with insulin resistance. Numerous studies have been carried out that demonstrate that IGT individuals with normal FPG values are at increased risk for cardiovascular disease. Patients with normal FPG values may have abnormal postprandial glucose values and are often unaware of their risk for pre-diabetes, diabetes, and cardiovascular disease.

[0078] "Fasting plasma glucose (FPG) test" is a simple test measuring blood glucose levels after an 8 hour fast. According to the ADA, blood glucose concentration of 100-125 mg/dL is considered IFG and defines pre-diabetes whereas $\geq$126 mg/dL defines diabetes. As stated by the ADA, FPG is the preferred test to diagnose diabetes and pre-diabetes due to its ease of use, patient acceptability, lower cost, and relative reproducibility. The weakness in the FPG test is that patients are quite advanced toward Type 2 Diabetes before fasting glucose levels change.

[0079] "Oral glucose tolerance test (OGTT)", a dynamic measurement of glucose, is a postprandial measurement of a patient's blood glucose levels after oral ingestion of a 75 g glucose drink. Traditional measurements include a fasting blood sample at the beginning of the test, a one hour time point blood sample, and a 2 hour time point blood sample. A patient's blood glucose concentration at the 2 hour time point defines the level of glucose tolerance: Normal glucose tolerance (NGT) $\leq$140 mg/dL blood glucose;

Impaired glucose tolerance (IGT) = 140-199 mg/dL blood glucose; Diabetes ≥200 mg/dL blood glucose. As stated by the ADA, even though the OGTT is known to be more sensitive and specific at diagnosing pre-diabetes and diabetes, it is not recommended for routine clinical use because of its poor reproducibility and difficulty to perform in practice.

[0080] "Fasting insulin test" measures the circulating mature form of insulin in plasma. The current definition of hyperinsulinemia is difficult due to lack of standardization of insulin immunoassays, cross-reactivity to proinsulin forms, and no consensus on analytical requirements for the assays. Within-assay CVs range from 3.7%-39% and among-assay CVs range from 12%-66%. Therefore, fasting insulin is not commonly measured in the clinical setting and is limited to the research setting.

[0081] The "hyperinsulinemic euglycemic clamp (HI clamp)" is considered worldwide as the "gold standard" for measuring insulin resistance in patients. It is performed in a research setting, requires insertion of two catheters into the patient and the patient must remain immobilized for up to six hours. The HI clamp involves creating steady-state hyperinsulinemia by insulin infusion, along with parallel glucose infusion in order to quantify the required amount of glucose to maintain euglycemia (normal concentration of glucose in the blood; also called *normoglycemia*). The result is a measure of the insulin-dependent glucose disposal rate (Rd), measuring the peripheral uptake of glucose by the muscle (primarily) and adipose tissues. This rate of glucose uptake is notated by M, whole body glucose metabolism by insulin action under steady state conditions. Therefore, a high M indicates high insulin sensitivity and a lower M value indicates reduced insulin sensitivity, i.e. insulin resistance. The HI clamp requires three trained professionals to carry out the procedure, including simultaneous infusions of insulin and glucose over 2-4 hours and frequent blood sampling every 5 minutes for analysis of insulin and glucose levels. Due to the high cost, complexity, and time required for the HI clamp, this procedure is strictly limited to the clinical research setting.

[0082] "Obesity" refers to a chronic condition defined by an excess amount body fat. The normal amount of body fat (expressed as percentage of body weight) is between 25-30% in women and 18-23% in men. Women with over 30% body fat and men with over 25% body fat are considered obese.

[0083] "Body Mass Index, (or BMI)" refers to a calculation that uses the height and weight of an individual to estimate the amount of the individual's body fat. Too much body fat (e.g. obesity) can lead to illnesses and other health problems. BMI is the measurement of choice for many physicians and researchers studying obesity. BMI is calculated using a mathematical formula that takes into account both height and weight of the individual. BMI equals a person's weight in kilograms divided by height in meters squared. (BMI=kg/m^2). Subjects having a BMI less than 19 are considered to be underweight, while those with a BMI of between 19 and 25 are considered to be of normal weight, while a BMI of between 25 to 29 are generally considered overweight, while individuals with a BMI of 30 or more are typically considered obese. Morbid obesity refers to a subject having a BMI of 40 or greater.

[0084] "Cardiovascular disease" refers to any disease of the heart or blood vessels. Cardiovascular or heart disease includes but is not limited to, for example, angina, arrhythmia, coronary artery disease (CAD), coronary heart disease, cardiomyopathy (including dilated cardiomyopathy, restrictive cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and diabetic cardiomyopathy) heart attack (myocardial infarction), heart failure, hypertrophic cardiomyopathy, mitral regurgitation, mitral valve prolapse, pulmonary stenosis, etc. Blood vessel disease includes but is not limited to, for example, peripheral vascular disease, artery disease, carotid artery disease, deep vein thrombosis, venous diseases, atherosclerosis, etc.

I. Biomarkers

[0085] The biomarkers described herein were discovered using metabolomic profiling techniques. Such metabolomic profiling techniques are described in more detail in the Examples set forth below as well as in U.S. Patents No. 7,005,255 and 7,329,489 and U.S. Patent Application Nos. 11/357,732 (Publication No. 2007/0026389), 11/301,077 (Publication No. 2006/0134676), 11/301,078 (Publication No. 2006/0134677), 11/301,079 (Publication No. 2006/0134678), and 11/405,033 (Publication No. US 2007/0072203), the entire contents of which are hereby incorporated herein by reference.

[0086] Generally, metabolic profiles may be determined for biological samples from human subjects diagnosed with a condition such as pre-diabetes as well as from one or

more other groups of human subjects (e.g., healthy control subjects with normal glucose tolerance, subjects with impaired glucose tolerance, subjects with insulin resistance). The metabolic profile for a pre-diabetes disorder may then be compared to the metabolic profile for biological samples from the one or more other groups of subjects. The comparisons may be conducted using models or algorithms, such as those described herein. Those molecules differentially present, including those molecules differentially present at a level that is statistically significant, in the metabolic profile of samples from subjects with a pre-diabetes disorder as compared to another group (e.g., healthy control subjects not pre-diabetic) may be identified as biomarkers to distinguish those groups.

[0087] Biomarkers for use in the methods disclosed herein may be obtained from any source of biomarkers related to pre-diabetes and/or type-2 diabetes. Biomarkers for use in methods disclosed herein relating to pre-diabetes include those listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, 29, and combinations and subsets thereof. In one embodiment, the biomarkers include those listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, and combinations thereof. Additional biomarkers include those disclosed in U.S. Application No. 60/950,286, the entirety of which is hereby incorporated by reference in its entirety. In one aspect, the biomarkers correlate to insulin resistance.

[0088] Biomarkers for use in methods disclosed herein relating to metabolic syndrome-related metabolic disorders include those listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 12, 13, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, 29, and combinations thereof. For example, biomarkers for use in distinguishing, or aiding in distinguishing, between subjects having metabolic syndrome and subjects not having metabolic syndrome include those biomarkers identified in Tables 4, 5, 6, 7, 8, 9A, 9B, 12, 13, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, 29, and combinations thereof. In one aspect, biomarkers for use in methods relating to metabolic syndrome include one or more of those listed in Tables 12 and 13. In another aspect biomarkers for use in methods relating to metabolic syndrome using plasma samples obtained from a subject include one or more of those listed in Table 12. In a preferred aspect, the biomarkers for use in methods disclosed herein related to metabolic syndrome using plasma samples obtained from a subject include one or more of the biomarkers N-acetylglycine, metabolite-6346, metabolite-8792, gamma-glu-leu, metabolite-4806, metabolite-3165, metabolite-7762, metabolite-3030,

metabolite-5978, metabolite-3218, metabolite-2000, metabolite-5848, metabolite-3370, malic acid, metabolite-3843, metabolite-4275, metabolite-3094, metabolite-4167, metabolite-3073, aldosterone, metabolite-1320, metabolite-2185, phenylalanine, metabolite-2139, glutamic acid, alpha-tocopherol, metabolite-5767, metabolite-5346, metabolite-9855, and 1-octadecanol, and combinations thereof. In yet another aspect, biomarkers for use in methods relating to metabolic syndrome using serum samples obtained from a subject include one or more of those listed in Table 13. In a preferred aspect, the biomarkers for use in metabolic syndrome methods disclosed herein using serum samples obtained from a subject include one or more of the biomarkers metabolite-8792, metabolite-5767, metabolite-2139, metabolite-8402, metabolite-3073, phenylalanine, metabolite-4929, metabolite-3370, nonanate, N-acetylglycine, metabolite-5848, metabolite-3077, monopalmitin, dioctyl-phthalate, octadecanoic acid, cholesterol, metabolite-2608, metabolite-6272, metabolite-3012, D-glucose, metabolite-2986, metabolite-4275, metabolite-6268, tyrosine, metabolite-10683, metabolite-2000, alpha-tocopherol, metabolite-2469, xanthine, and metabolite-2039, and combinations thereof.

[0089] In another aspect, biomarkers for use in methods disclosed herein relating to metabolic syndrome may include the use of one or more biomarkers listed in Tables 12 and/or 13 in combination with one or more biomarkers in one or more of Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, or combinations thereof. For example, biomarkers for use in methods relating to metabolic syndrome may include one or more biomarkers listed in Tables 12 and/or 13 in combination with one or more biomarkers associated with insulin resistance, such as those listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, or combinations thereof.

[0090] Biomarkers for use in methods disclosed herein relating to pre-diabetic or diabetic conditions, such as impaired insulin sensitivity, insulin resistance, or type-2 diabetes include one or more of those listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, and combinations thereof. Such biomarkers allow subjects to be classified as insulin resistant, insulin impaired, or insulin sensitive. In one aspect, biomarkers for use in distinguishing or aiding in distinguishing, between subjects having impaired insulin sensitivity from subjects not having impaired insulin sensitivity include one or more of those listed in Table 4, 5, 6, 7, 8, 9A, and/or 9B. In another aspect, biomarkers for use in

diagnosing insulin resistance include one or more of those listed in Tables 4, 5, 6, 7, 8, 9A, and/or 9B. In another example, biomarkers for use in distinguishing subjects having insulin resistance from subject not having insulin resistance include one or more of those listed in Tables 4, 5, 6, 7, 8, 9A, and/or 9B. In another example, biomarkers for use in categorizing, or aiding in categorizing, a subject as having impaired fasting glucose levels or impaired glucose tolerance include one or more of those listed in Tables 4, 5, 6, 7, 8, 9A, and/or 9B.

[0091] Biomarkers for use in methods disclosed herein relating to atherosclerosis include one or more of those listed in Tables 14, 15, 16, and/or 17 and combinations thereof. For example, biomarkers for use in distinguishing, or aiding in distinguishing, atherosclerotic subjects from non-atherosclerotic subjects include one or more of those biomarkers listed in Tables 14, 15, 16, 17, 3-methylhistidine, p-cresol sulfate, mannose, glucose, and/or gluconate, and combinations thereof. In one aspect biomarkers for use in methods relating to atherosclerosis using plasma samples from a subject include one or more of 3-methylhistidine, p-cresol sulfate, mannose, glucose, gluconate, and those listed in Tables 14 and 17. In another aspect biomarkers for use in methods relating to atherosclerosis using aortic samples from a subject include one or more of those listed in Table 15. In yet another aspect, biomarkers for use in methods relating to atherosclerosis using liver samples from a subject include one or more of those listed in Table 16. In one aspect, preferred biomarkers for use in methods involving subjects in an early stage of atherosclerosis include the biomarkers identified in Figures 10A, 11A, and 12A. Preferred biomarkers for use in methods involving subjects in a mid-stage of atherosclerosis include the biomarkers identified in Figures 10B, 11B, and 12B. Preferred biomarkers for use in methods involving subjects in a later stage of atherosclerosis include the biomarkers identified in Figures 10C, 11C, and 12C. Preferred biomarkers for use in methods involving subjects in any stage of atherosclerosis include the biomarkers identified in Figures 10D, 11D, and 12D.

[0092] Biomarkers for use in methods disclosed herein relating to cardiomyopathy include one or more of those biomarkers listed in Tables 21, 22, 23, and/or 25. Such markers may be used, for example, to distinguish, or aiding in distinguishing, between subjects having cardiomyopathy from subjects not having cardiomyopathy. In one

aspect, biomarkers for use in methods relating to cardiomyopathy using cardiac tissue samples from a subject include one or more of those listed in Table 21. In another aspect, biomarkers for use in methods relating to cardiomyopathy using plasma samples from a subject include one or more of those listed in Table 22 and/or 23.

[0093] Biomarkers for use in methods disclosed herein relating to obesity include one or more of those biomarkers listed in Table 26. Such markers may be used, for example, to distinguish obese subjects from lean subjects. Such markers may also be used in combination with biomarkers for pre-diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy. In another aspect, the markers may be used, for example, to determine susceptibility to obesity or weight gain. In another aspect, the markers may be used, for example, to determine if a therapeutic agent is likely to induce weight gain in a subject.

[0094] Any number of biomarkers may be used in the methods disclosed herein. That is, the disclosed methods may include the determination of the level(s) of one biomarker, two or more biomarkers, three or more biomarkers, four or more biomarkers, five or more biomarkers, six or more biomarkers, seven or more biomarkers, eight or more biomarkers, nine or more biomarkers, ten or more biomarkers, fifteen or more biomarkers, etc., including a combination of all of the biomarkers in each or all of Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, 29, or any fraction thereof. In another aspect, the number of biomarkers for use in the disclosed methods include the levels of about thirty or less biomarkers, twenty-five or less, twenty or less, fifteen or less, ten or less, nine or less, eight or less, seven or less, six or less, five or less biomarkers. In another aspect, the number of biomarkers for use in the disclosed methods include the levels of one, two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, twenty, twenty-five, or thirty biomarkers.

[0095] Although the identities of some of the biomarkers compounds are not known at this time, such identities are not necessary for the identification of the biomarkers in biological samples from subjects, as the "unnamed" compounds have been sufficiently characterized by analytical techniques to allow such identification. The analytical characterization of all such "unnamed" compounds is listed in the Examples. Such "unnamed" biomarkers are designated herein using the nomenclature "Metabolite" followed by a specific metabolite number.

[0096] In addition, the methods disclosed herein using the biomarkers listed in the tables may be used in combination with clinical diagnostic measures of the respective conditions. Combinations with clinical diagnostics may facilitate the disclosed methods, or confirm results of the disclosed methods (for example, facilitating or confirming diagnosis, monitoring progression or regression, and/or determining predisposition to pre-diabetes).

[0097] Finally, where the potential identity of a compound is proposed for an "unnamed" metabolite and such identity has not been confirmed, the nomenclature of "possible" (along with the potential compound identity) follows the "Metabolite" number. Such proposed identity should not be considered as limiting the analytical characterization of the otherwise "unnamed" compounds.

II. Diagnostic Methods

[0098] The biomarkers described herein may be used to diagnose, or to aid in diagnosing, whether a subject has a disease or condition, such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy. For example, biomarkers for use in diagnosing, or aiding in diagnosing, whether a subject has a insulin resistance include one or more of those identified in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, 29, and combinations thereof. In one embodiment, the biomarkers include one or more of those identified in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, and combinations thereof. In another embodiment, combinations of biomarkers include those, such as 2-hydroxybutyrate in combination with one or more biomarkers indentified in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, and/or 29.

[0099] Methods for diagnosing, or aiding in diagnosing, whether a subject has a disease or condition, such as pre-diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, may performed using one or more of the biomarkers identified in the respective tables provided herein. A method of diagnosing (or aiding in diagnosing) whether a subject has a disease or condition, such as pre-diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, comprises (1) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers of the disease or condition in the sample and (2) comparing the level(s) of one or more biomarkers in the sample to disease- or condition-positive and/or disease- or condition-negative reference levels of

the one or more biomarkers to diagnose (or aid in the diagnosis of) whether the subject has the disease or condition. For example, a method of diagnosing (or aiding in diagnosing) whether a subject is pre-diabetic comprises (1) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers of pre-diabetes in the sample and (2) comparing the level(s) of the one or more biomarkers in the sample to pre-diabetes-positive and/or pre-diabetes-negative reference levels of the one or more biomarkers in order to diagnose (or aid in the diagnosis of) whether the subject has pre-diabetes. The one or more biomarkers that are used are selected from Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof. When such a method is used in aiding in the diagnosis of a disease or condition, such as insulin resistance, pre-diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, the results of the method may be used along with other methods (or the results thereof) useful in the clinical determination of whether a subject has a given disease or condition. Methods useful in the clinical determination of whether a subject has a disease or condition such as pre-diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy are known in the art. For example, methods useful in the clinical determination of whether a subject has pre-diabetes include, for example, glucose disposal rates (Rd), body weight measurements, waist circumference measurements, BMI determinations, Peptide YY measurements, Hemoglobin A1C measurements, adiponectin measurements, fasting plasma glucose measurements, free fatty acid measurements, fasting plasma insulin measurements, and the like. Methods useful for the clinical determination of atherosclerosis and/or cardiomyopathy in a subject include angiography, stress-testing, blood tests (e.g. to measure homocysteine, fibrinogen, lipoprotein (a), small LDL particles, and c-reactive protein levels), electrocardiography, echocardiography, computed tomography (CT) scans, ankle/brachial index, and intravascular ultrasounds.

[00100] In another example, the identification of biomarkers for diseases or conditions such as insulin resistance, pre-diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy, allows for the diagnosis of (or for aiding in the diagnosis of) such diseases or conditions in subjects presenting one or more symptoms of the disease or condition. For example, a method of diagnosing (or aiding in diagnosing) whether a subject has insulin resistance comprises (1) analyzing a biological sample from a subject

to determine the level(s) of one or more biomarkers of insulin resistance in the sample and (2) comparing the level(s) of the one or more biomarkers in the sample to insulin resistance-positive and/or insulin resistance-negative reference levels of the one or more biomarkers in order to diagnose (or aid in the diagnosis of) whether the subject has insulin resistance. The one or more biomarkers that are used are selected from Tables 4, 5, 6, 7, 8, 9A, and/or 9B. The biomarkers for insulin resistance may also be used to classify subjects as being either insulin resistant, insulin sensitive, or having impaired insulin sensitivity. As described in Example 2, below, biomarkers are identified that may be used to classify subjects as being insulin resistant, insulin sensitive, or having impaired insulin sensitivity. The biomarkers in Tables 4, 5, 6, 7, 8, 9A, and/or 9B, may also be used to classify subjects as having impaired fasting glucose levels or impaired glucose tolerance or normal glucose tolerance. Thus, the biomarkers may indicate compounds that increase and decrease as the glucose disposal rate increases. By determining appropriate reference levels of the biomarkers for each group (insulin resistant, insulin impaired, insulin sensitive), subjects can be diagnosed appropriately. The results of this method may be combined with the results of clinical measurements to aid in the diagnosis of insulin resistance or for categorizing the subject as having NGT, IFG, or IGT.

[00101] Increased insulin resistance correlates with the glucose disposal rate (Rd) as measured by the HI clamp. As exemplified below, metabolomic analysis was carried out to identify biomarkers that correlate with the glucose disposal rate (Rd). These biomarkers can be used in a mathematical model to determine the glucose disposal rate of the subject. The insulin sensitivity of the individual can be determined using this model. Using metabolomic analysis, panels of metabolites that can be used in a simple blood test to predict insulin resistance as measured by the "gold standard" of hyperinsulinemic euglycemic clamps in at least two independent cohorts of subjects were discovered. In another example, biomarkers are identified that correlate with the results of oral glucose tolerance tests (OGTT) for use in categorizing subjects as having normal glucose tolerance (NGT), impaired fasting glucose levels (IFG), or impaired glucose tolerance (IGT).

[00102] Independent studies were carried out to identify a set of biomarkers that when

used with a polynomic algorithm will enable the early detection of changes in insulin resistance in a subject. In one aspect, the instant invention provides the subject with a score indicating the level of insulin resistance ("IR Score") of the subject. The score is based upon clinically significant changed reference level for a biomarker and/or combination of biomarkers. The reference level can be derived from an algorithm or computed from indices for impaired glucose tolerance and can be presented in a report as shown in Figure 5. The IR Score places the subject in the range of insulin resistance from normal (i.e. insulin sensitive) to high. Disease progression or remission can be monitored by periodic determination and monitoring of the IR Score. Response to therapeutic intervention can be determined by monitoring the IR Score. The IR Score can also be used to evaluate drug efficacy.

[00103] Methods for determining a subject's insulin resistance score (IR score) may be performed using one or more of the biomarkers identified in the respective Tables provided herein. For example, a method for determining the IR score of a subject comprises the steps of: (1) analyzing a biological sample from a subject to determine the level(s) of one or more insulin resistance biomarkers in the sample, and (2) comparing the level(s) of the one or more insulin resistance biomarkers in the sample to insulin resistance reference levels of the one or more biomarkers in order to determine the subject's insulin resistance score. The one or more biomarkers that are used may be selected from Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof. The method may employ any number of markers selected from Tables 4, 5, 6, 7, 8, 9A, and/or 9B, including 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more markers. Multiple biomarkers may be correlated with a given condition, such as insulin resistance, by any method, including statistical methods such as regression analysis.

[00104] Also as exemplified below, metabolomic analysis was carried out to identify biomarkers that correlate with metabolic syndrome, atherosclerosis, cardiomyopathy, and other diseases or conditions. Such biomarkers may be used in the methods of the present invention to analyze biological samples to identify or measure the level of the biomarkers in the sample.

[00105] Any suitable method may be used to analyze the biological sample in order to determine the level(s) of the one or more biomarkers in the sample. Suitable methods

include chromatography (e.g., HPLC, gas chromatography, liquid chromatography), mass spectrometry (e.g., MS, MS-MS), enzyme-linked immunosorbent assay (ELISA), antibody linkage, other immunochemical techniques, and combinations thereof. Further, the level(s) of the one or more biomarkers may be measured indirectly, for example, by using an assay that measures the level of a compound (or compounds) that correlates with the level of the biomarker(s) that are desired to be measured.

[00106] After the level(s) of the one or more biomarker(s) is determined, the level(s) may be compared to disease or condition reference level(s) of the one or more biomarker(s) to determine a rating for each of the one or more biomarker(s) in the sample. The rating(s) may be aggregated using any algorithm to create a score, for example, an insulin resistance (IR) score, for the subject. The algorithm may take into account any factors relating to the disease or condition, such as insulin resistance, including the number of biomarkers, the correlation of the biomarkers to the disease or condition, etc.

[00107] In one example, the subject's insulin resistance score may be correlated to any index indicative of a level insulin resistance, from normal glucose tolerance to insulin resistant. For example, a subject having an insulin resistance score of less than 25 may indicate that the subject has normal glucose tolerance; a score of between 26 and 50 may indicate that the subject has low impaired glucose tolerance; a score of between 51 and 75 may indicate that the subject has medium impaired glucose tolerance; a score of between 76 and 100 may indicate that the subject has high impaired glucose tolerance; and a score above 100 may indicate that the subject has type-2 diabetes.

III. Monitoring Disease or Condition Progression / Regression

[00108] The identification of biomarkers herein allows for monitoring progression/regression of the respective diseases or conditions (e.g. pre-diabetes, metabolic syndrome, atherosclerosis, cardiomyopathy, insulin resistance, etc.) in a subject. A method of monitoring the progression/regression of disease or condition, such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy, in a subject comprises (1) analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for the respective disease or condition selected from Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27,

28, and combinations thereof in the first sample obtained from the subject at a first time point, (2) analyzing a second biological sample from a subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point, and (3) comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample in order to monitor the progression/regression of the disease or condition in the subject. The results of the method are indicative of the course of the disease or condition (i.e., progression or regression, if any change) in the subject.

[00109] In one embodiment, the results of the method may be based on Insulin Resistance (IR) Score which is indicative of the insulin resistance in the subject and which can be monitored over time. By comparing the IR Score from a first time point sample to the IR Score from at least a second time point sample the progression or regression of IR can be determined. Such a method of monitoring the progression/regression of pre-diabetes and/or type-2 diabetes in a subject comprises (1) analyzing a first biological sample from a subject to determine an IR score for the first sample obtained from the subject at a first time point, (2) analyzing a second biological sample from a subject to determine a second IR score, the second sample obtained from the subject at a second time point, and (3) comparing the IR score in the first sample to the IR score in the second sample in order to monitor the progression/regression of pre-diabetes and/or type-2 diabetes in the subject.

[00110] Using the biomarkers and algorithm of the instant invention for progression monitoring may guide, or assist a physician's decision to implement preventative measures such as dietary restrictions, exercise, or early-stage drug treatment.

IV. Determining Predisposition to a Disease or Condition

[00111] The biomarkers identified herein may also be used in the determination of whether a subject not exhibiting any symptoms of a disease or condition, such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy. The biomarkers may be used, for example, to determine whether a subject is predisposed to developing, for example, insulin resistance. Such methods of determining whether a subject having no symptoms of a particular disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, is predisposed

to developing a particular disease or condition comprise (1) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers listed in the respective tables (e.g. Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, and combinations thereof) in the sample and (2) comparing the level(s) of the one or more biomarkers in the sample to disease- or condition-positive and/or disease- or condition-negative reference levels of the one or more biomarkers in order to determine whether the subject is predisposed to developing the respective disease or condition. For example, the identification of biomarkers for insulin resistance allows for the determination of whether a subject having no symptoms of insulin resistance is predisposed to developing insulin resistance. A method of determining whether a subject having no symptoms of insulin resistance is predisposed to developing insulin resistance comprises (1) analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, and 9B, and combinations thereof in the sample and (2) comparing the level(s) of the one or more biomarkers in the sample to insulin resistance-positive and/or insulin resistance-negative reference levels of the one or more biomarkers in order to determine whether the subject is predisposed to developing insulin resistance. The results of the method may be used along with other methods (or the results thereof) useful in the clinical determination of whether a subject is predisposed to developing the disease or condition.

[00112] After the level(s) of the one or more biomarkers in the sample are determined, the level(s) are compared to disease- or condition-positive and/or disease- or condition-negative reference levels in order to predict whether the subject is predisposed to developing a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy. Levels of the one or more biomarkers in a sample corresponding to the disease- or condition-positive reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the subject being predisposed to developing the disease or condition. Levels of the one or more biomarkers in a sample corresponding to disease- or condition-negative reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or

below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the subject not being predisposed to developing the disease or condition. In addition, levels of the one or more biomarkers that are differentially present (especially at a level that is statistically significant) in the sample as compared to disease- or condition-negative reference levels may be indicative of the subject being predisposed to developing the disease or condition. Levels of the one or more biomarkers that are differentially present (especially at a level that is statistically significant) in the sample as compared to disease- condition-positive reference levels are indicative of the subject not being predisposed to developing the disease or condition.

[00113] By way of example, after the level(s) of the one or more biomarkers in the sample are determined, the level(s) are compared to insulin resistance-positive and/or insulin resistance-negative reference levels in order to predict whether the subject is predisposed to developing insulin resistance. Levels of the one or more biomarkers in a sample corresponding to the insulin resistance-positive reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the subject being predisposed to developing insulin resistance. Levels of the one or more biomarkers in a sample corresponding to the insulin resistance-negative reference levels (e.g., levels that are the same as the reference levels, substantially the same as the reference levels, above and/or below the minimum and/or maximum of the reference levels, and/or within the range of the reference levels) are indicative of the subject not being predisposed to developing insulin resistance. In addition, levels of the one or more biomarkers that are differentially present (especially at a level that is statistically significant) in the sample as compared to insulin resistance-negative reference levels are indicative of the subject being predisposed to developing insulin resistance. Levels of the one or more biomarkers that are differentially present (especially at a level that is statistically significant) in the sample as compared to insulin resistance-positive reference levels are indicative of the subject not being predisposed to developing insulin resistance. Although insulin resistance is discussed in this example, predisposition to the other diseases or conditions may also be determined in accordance with this method by using one or more of the respective

biomarkers as set forth above.

[00114] Furthermore, it may also be possible to determine reference levels specific to assessing whether or not a subject that does not have a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, is predisposed to developing a disease or condition. For example, it may be possible to determine reference levels of the biomarkers for assessing different degrees of risk (e.g., low, medium, high) in a subject for developing a disease or condition. Such reference levels could be used for comparison to the levels of the one or more biomarkers in a biological sample from a subject.

V. Monitoring Therapeutic Efficacy:

[00115] The biomarkers provided also allow for the assessment of the efficacy of a composition for treating a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy. For example, the identification of biomarkers for insulin resistance also allows for assessment of the efficacy of a composition for treating insulin resistance as well as the assessment of the relative efficacy of two or more compositions for treating insulin resistance. Such assessments may be used, for example, in efficacy studies as well as in lead selection of compositions for treating the disease or condition.

[00116] Thus, also provided are methods of assessing the efficacy of a composition for treating a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy comprising (1) analyzing, from a subject (or group of subjects) having a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy and currently or previously being treated with a composition, a biological sample (or group of samples) to determine the level(s) of one or more biomarkers for the disorder selected from Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and combinations thereof, and (2) comparing the level(s) of the one or more biomarkers in the sample to (a) level(s) of the one or more biomarkers in a previously-taken biological sample from the subject, wherein the previously-taken biological sample was obtained from the subject before being treated with the composition, (b) disease- or condition-positive reference levels of the one or more biomarkers, (c) disease- or condition-negative reference levels of the one

or more biomarkers, (d) disease- or condition-progression-positive reference levels of the one or more biomarkers, and/or (e) disease- or condition-regression-positive reference levels of the one or more biomarkers. The results of the comparison are indicative of the efficacy of the composition for treating the respective disease or condition.

[00117] The change (if any) in the level(s) of the one or more biomarkers over time may be indicative of progression or regression of the disease or condition in the subject. To characterize the course of a given disease or condition in the subject, the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples may be compared to the respective disease- or condition-positive and/or disease- or condition-negative reference levels of the one or more biomarkers. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to the disease- or condition-positive reference levels (or less similar to the disease- or condition-negative reference levels), then the results are indicative of the disease's or condition's progression. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to the disease- or condition-negative reference levels (or less similar to the disease- or condition-positive reference levels), then the results are indicative of the disease's or condition's regression.

[00118] For example, in order to characterize the course of insulin resistance in the subject, the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples may be compared to insulin resistance-positive and/or insulin resistance-negative reference levels of the one or more biomarkers. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to the insulin resistance-positive reference levels (or less similar to the insulin resistance-negative reference levels), then the results are indicative of insulin resistance progression. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to the

insulin resistance-negative reference levels (or less similar to the insulin resistance-positive reference levels), then the results are indicative of insulin resistance regression.

[00119] The second sample may be obtained from the subject any period of time after the first sample is obtained. In one aspect, the second sample is obtained 1, 2, 3, 4, 5, 6, or more days after the first sample or after the initiation of the administration of a composition. In another aspect, the second sample is obtained 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more weeks after the first sample or after the initiation of the administration of a composition. In another aspect, the second sample may be obtained 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months after the first sample or after the initiation of the administration of a composition.

[00120] The course of a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy in a subject may also be characterized by comparing the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples to disease- or condition-progression-positive and/or disease- or condition-regression-positive reference levels. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to the disease- or condition-progression-positive reference levels (or less similar to the disease- or condition-regression-positive reference levels), then the results are indicative of the disease or condition progression. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to the disease- or condition-regression-positive reference levels (or less similar to the disease- or condition-progression-positive reference levels), then the results are indicative of disease or condition regression.

[00121] As with the other methods described herein, the comparisons made in the methods of monitoring progression/regression of a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy in a subject may be carried out using various techniques, including simple comparisons, one or more statistical analyses, and combinations thereof.

[00122] The results of the method may be used along with other methods (or the results thereof) useful in the clinical monitoring of progression/regression of the disease or condition in a subject.

[00123] As described above in connection with methods of diagnosing (or aiding in the diagnosis of) a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, any suitable method may be used to analyze the biological samples in order to determine the level(s) of the one or more biomarkers in the samples. In addition, the level(s) one or more biomarkers, including a combination of all of the biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and/or 28, or any fraction thereof, may be determined and used in methods of monitoring progression/regression of the respective disease or condition in a subject.

[00124] Such methods could be conducted to monitor the course of disease or condition development in subjects, for example the course of pre-diabetes to type-2 diabetes in a subject having pre-diabetes, or could be used in subjects not having a disease or condition (e.g., subjects suspected of being predisposed to developing the disease or condition) in order to monitor levels of predisposition to the disease or condition.

[00125] Clinical studies from around the world have been carried out to test whether anti-diabetic therapies, such as metformin or acarbose, can prevent diabetes progression in pre-diabetic patients. These studies have shown that such therapies can prevent diabetes onset. From the U.S. Diabetes Prevention Program (DPP), metformin reduced the rate of progression to diabetes by 38% and lifestyle and exercise intervention reduced the rate of progression to diabetes by 56%. Because of such successes, the ADA has revised its 2008 Standards of Medical Care in Diabetes to include the following statements in the section on Prevention/Delay of Type 2 Diabetes: *"In addition to lifestyle counseling, metformin may be considered in those who are at very high risk (combined IFG and IGT plus other risk factors) and who are obese and under 60 years of age."*

[00126] Pharmaceutical companies have carried out studies to assess whether certain classes of drugs, such as the PPARγ class of insulin sensitizers, can prevent diabetes

progression. Similar to the DPP trial, some of these studies have shown great promise and success for preventing diabetes, whereas others have exposed a certain amount of risk associated with certain anti-diabetic pharmacologic treatments when given to the general pre-diabetic population as defined by current IR diagnostics. Pharmaceutical companies are in need of diagnostics that can identify and stratify high risk pre-diabetics so they can assess the efficacy of their pre-diabetic therapeutic candidates more effectively and safely.

[00127] Considering the infrequency of the oral glucose tolerance test (OGTT) procedures in the clinical setting, a new diagnostic test that directly measures insulin resistance in a fasted sample would enable a physician to identify and stratify patients who are moving toward the etiology of pre-diabetes and cardiovascular disease much earlier.

VI. Identification of Responders and Non-responders to Therapeutic:

[00128] The biomarkers provided also allow for the identification of subjects in whom the composition for treating a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy is efficacious (i.e. patient responds to therapeutic). For example, the identification of biomarkers for insulin resistance also allows for assessment of the subject response to a composition for treating insulin resistance as well as the assessment of the relative patient response to two or more compositions for treating insulin resistance. Such assessments may be used, for example, in selection of compositions for treating the disease or condition for certain subjects.

[00129] Thus, also provided are methods of predicting the response of a patient to a composition for treating a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy comprising (1) analyzing, from a subject (or group of subjects) having a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy and currently or previously being treated with a composition, a biological sample (or group of samples) to determine the level(s) of one or more biomarkers for the disorder selected from Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and 28, and combinations thereof, and (2) comparing the level(s) of the one or more biomarkers in the sample to (a) level(s) of the one or more biomarkers in a previously-taken biological sample from the subject,

wherein the previously-taken biological sample was obtained from the subject before being treated with the composition, (b) disease- or condition-positive reference levels of the one or more biomarkers, (c) disease- or condition-negative reference levels of the one or more biomarkers, (d) disease- or condition-progression-positive reference levels of the one or more biomarkers, and/or (e) disease- or condition-regression-positive reference levels of the one or more biomarkers. The results of the comparison are indicative of the response of the patient to the composition for treating the respective disease or condition.

[00130] The change (if any) in the level(s) of the one or more biomarkers over time may be indicative of response of the subject to the therapeutic. To characterize the course of a given therapeutic in the subject, the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples may be compared to the respective disease- or condition-positive and/or disease- or condition-negative reference levels of the one or more biomarkers. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to the disease- or condition-positive reference levels (or less similar to the disease- or condition-negative reference levels), then the results are indicative of the patient not responding to the therapeutic. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to the disease- or condition-negative reference levels (or less similar to the disease- or condition-positive reference levels), then the results are indicative of the patient responding to the therapeutic.

[00131] For example, in order to characterize the patient response to a therapeutic for insulin resistance, the level(s) of the one or more biomarkers in the first sample, the level(s) of the one or more biomarkers in the second sample, and/or the results of the comparison of the levels of the biomarkers in the first and second samples may be compared to insulin resistance-positive and/or insulin resistance-negative reference levels of the one or more biomarkers. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time (e.g., in the second sample as compared to the first sample) to become more similar to the insulin resistance-positive

reference levels (or less similar to the insulin resistance-negative reference levels), then the results are indicative of non-response to the therapeutic. If the comparisons indicate that the level(s) of the one or more biomarkers are increasing or decreasing over time to become more similar to the insulin resistance-negative reference levels (or less similar to the insulin resistance-positive reference levels), then the results are indicative of response to the therapeutic.

[00132] The second sample may be obtained from the subject any period of time after the first sample is obtained. In one aspect, the second sample is obtained 1, 2, 3, 4, 5, 6, or more days after the first sample. In another aspect, the second sample is obtained 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more weeks after the first sample or after the initiation of treatment with the composition. In another aspect, the second sample may be obtained 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or more months after the first sample or after the initiation of treatment with the composition.

[00133] As with the other methods described herein, the comparisons made in the methods of determining a patient response to a therapeutic for a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy in a subject may be carried out using various techniques, including simple comparisons, one or more statistical analyses, and combinations thereof.

[00134] The results of the method may be used along with other methods (or the results thereof) useful in determining a patient response to a therapeutic for the disease or condition in a subject.

[00135] As described above in connection with methods of diagnosing (or aiding in the diagnosis of) a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, any suitable method may be used to analyze the biological samples in order to determine the level(s) of the one or more biomarkers in the samples. In addition, the level(s) one or more biomarkers, including a combination of all of the biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and/or 28, or any fraction thereof, may be determined and used in methods of monitoring progression/regression of the respective disease or condition in a subject.

[00136] Such methods could be conducted to monitor the patient response to a therapeutic for a disease or condition development in subjects, for example the course of

pre-diabetes to type-2 diabetes in a subject having pre-diabetes, or could be used in subjects not having a disease or condition (e.g., subjects suspected of being predisposed to developing the disease or condition) in order to monitor levels of predisposition to the disease or condition.

[00137] Pharmaceutical companies have carried out studies to assess whether certain classes of drugs, such as the PPARγ class of insulin sensitizers, can prevent diabetes progression. Some of these studies have shown great promise and success for preventing diabetes, whereas others have exposed a certain amount of risk associated with certain anti-diabetic pharmacologic treatments when given to the general pre-diabetic population as defined by current IR diagnostics. Pharmaceutical companies are in need of diagnostics that can identify responders and non-responders in order to stratify high risk pre-diabetics to assess the efficacy of their pre-diabetic therapeutic candidates more effectively and safely. A new diagnostic test that discriminates non-responding from responding patients to a therapeutic would enable pharmaceutical companies to identify and stratify patients that are likely to respond to the therapeutic agent and target specific therapeutics for certain cohorts that are likely to respond to the therapeutic.

VII. Methods of Screening a Composition for Activity in Modulating Biomarkers

[00138] The biomarkers provided herein also allow for the screening of compositions for activity in modulating biomarkers associated with a disease or condition, such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy, which may be useful in treating the disease or condition. Such methods comprise assaying test compounds for activity in modulating the levels of one or more biomarkers selected from the respective biomarkers listed in the respective tables. Such screening assays may be conducted in vitro and/or in vivo, and may be in any form known in the art useful for assaying modulation of such biomarkers in the presence of a test composition such as, for example, cell culture assays, organ culture assays, and in vivo assays (e.g., assays involving animal models). For example, the identification of biomarkers for insulin resistance also allows for the screening of compositions for activity in modulating biomarkers associated with insulin resistance, which may be useful in treating insulin

resistance. Methods of screening compositions useful for treatment of insulin resistance comprise assaying test compositions for activity in modulating the levels of one or more biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, and/or 28. Although insulin resistance is discussed in this example, the other diseases and conditions such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy may also be diagnosed or aided to be diagnosed in accordance with this method by using one or more of the respective biomarkers as set forth above.

[00139] The methods for screening a composition for activity in modulating one or more biomarkers of a disease or condition such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy comprise (1) contacting one or more cells with a composition, (2) analyzing at least a portion of the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more biomarkers of a disease or condition selected from the biomarkers provided in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, and/or 26; and (3) comparing the level(s) of the one or more biomarkers with predetermined standard levels for the one or more biomarkers to determine whether the composition modulated the level(s) of the one or more biomarkers. In one embodiment, a method for screening a composition for activity in modulating one or more biomarkers of insulin resistance comprises (1) contacting one or more cells with a composition, (2) analyzing at least a portion of the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more biomarkers of insulin resistance selected from Tables 4, 5, 6, 7, 8, 9A, and/or 9B; and (3) comparing the level(s) of the one or more biomarkers with predetermined standard levels for the one or more biomarkers to determine whether the composition modulated the level(s) of the one or more biomarkers. As discussed above, the cells may be contacted with the composition in vitro and/or in vivo. The predetermined standard levels for the one or more biomarkers may be the levels of the one or more biomarkers in the one or more cells in the absence of the composition. The predetermined standard levels for the one or more biomarkers may also be the level(s) of the one or more biomarkers in control cells not contacted with the composition.

[00140] In addition, the methods may further comprise analyzing at least a portion of

the one or more cells or a biological sample associated with the cells to determine the level(s) of one or more non-biomarker compounds of a disease or condition, such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy. The levels of the non-biomarker compounds may then be compared to predetermined standard levels of the one or more non-biomarker compounds.

[00141] Any suitable method may be used to analyze at least a portion of the one or more cells or a biological sample associated with the cells in order to determine the level(s) of the one or more biomarkers (or levels of non-biomarker compounds). Suitable methods include chromatography (e.g., HPLC, gas chromatography, liquid chromatography), mass spectrometry (e.g., MS, MS-MS), ELISA, antibody linkage, other immunochemical techniques, biochemical or enzymatic reactions or assays, and combinations thereof. Further, the level(s) of the one or more biomarkers (or levels of non-biomarker compounds) may be measured indirectly, for example, by using an assay that measures the level of a compound (or compounds) that correlates with the level of the biomarker(s) (or non-biomarker compounds) that are desired to be measured.

VIII. Method of Identifying Potential Drug Targets

[00142] The disclosure also provides methods of identifying potential drug targets for diseases or conditions such as insulin resistance, pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy, using the biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and/or 28. A method for identifying a potential drug target for a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis and cardiomyopathy comprises (1) identifying one or more biochemical pathways associated with one or more biomarkers for a metabolic syndrome-related metabolic disorder selected from the respective tables (Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and/or 28); and (2) identifying a protein (e.g., an enzyme) affecting at least one of the one or more identified biochemical pathways, the protein being a potential drug target for the disease or condition. For example, the identification of biomarkers for insulin resistance also allows for the identification of potential drug targets for insulin resistance. A method for

identifying a potential drug target for insulin resistance comprises (1) identifying one or more biochemical pathways associated with one or more biomarkers for insulin resistance selected from Tables 4, 5, 6, 7, 8, 9A, 9B, 27, and/or 28, and (2) identifying a protein (e.g., an enzyme) affecting at least one of the one or more identified biochemical pathways, the protein being a potential drug target for insulin resistance. Although insulin resistance is discussed in this example, the other diseases or conditions such as type-2 diabetes, metabolic syndrome, atherosclerosis and cardiomyopathy, may also be diagnosed or aided to be diagnosed in accordance with this method by using one or more of the respective biomarkers as set forth above.

[00143] Another method for identifying a potential drug target for a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy comprises (1) identifying one or more biochemical pathways associated with one or more biomarkers for a metabolic syndrome-related metabolic disorder selected from the respective table(s) (Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, and/or 28) and one or more non-biomarker compounds of the disease or condition and (2) identifying a protein affecting at least one of the one or more identified biochemical pathways, the protein being a potential drug target for the disease or condition. For example, a method for identifying a potential drug target for insulin resistance comprises (1) identifying one or more biochemical pathways associated with one or more biomarkers for insulin resistance selected from Tables 4, 5, 6, 7, 8, 9A, 9B, 27, and/or 28, and one or more non-biomarker compounds of insulin resistance and (2) identifying a protein affecting at least one of the one or more identified biochemical pathways, the protein being a potential drug target for insulin resistance.

[00144] One or more biochemical pathways (e.g., biosynthetic and/or metabolic (catabolic) pathway) are identified that are associated with one or more biomarkers (or non-biomarker compounds). After the biochemical pathways are identified, one or more proteins affecting at least one of the pathways are identified. Preferably, those proteins affecting more than one of the pathways are identified.

[00145] A build-up of one metabolite (e.g., a pathway intermediate) may indicate the presence of a 'block' downstream of the metabolite and the block may result in a low/absent level of a downstream metabolite (e.g. product of a biosynthetic pathway). In

a similar manner, the absence of a metabolite could indicate the presence of a 'block' in the pathway upstream of the metabolite resulting from inactive or non-functional enzyme(s) or from unavailability of biochemical intermediates that are required substrates to produce the product. Alternatively, an increase in the level of a metabolite could indicate a genetic mutation that produces an aberrant protein which results in the over-production and/or accumulation of a metabolite which then leads to an alteration of other related biochemical pathways and result in dysregulation of the normal flux through the pathway; further, the build-up of the biochemical intermediate metabolite may be toxic or may compromise the production of a necessary intermediate for a related pathway. It is possible that the relationship between pathways is currently unknown and this data could reveal such a relationship.

[00146] The proteins identified as potential drug targets may then be used to identify compositions that may be potential candidates for treating a particular disease or condition, such as insulin resistance, including compositions for gene therapy.

IX. Methods of Treatment

[00147] In another aspect, methods for treating a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy are provided. The methods generally involve treating a subject having a disease or condition such as pre-diabetes, type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy with an effective amount of one or more biomarker(s) that are lowered in a subject having the disease or condition as compared to a healthy subject not having the disease or condition. The biomarkers that may be administered may comprise one or more of the biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 that are decreased in a disease or condition state as compared to subjects not having that disease or condition. Such biomarkers could be isolated based on the identity of the biomarker compound (i.e. compound name). The biomarkers that are currently unnamed metabolites could be isolated based on the analytical characterizations for the biomarkers listed in the Examples below (e.g. Table 29). In some embodiments, the biomarkers that are administered are one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29

that are decreased in a metabolic syndrome-related metabolic disorder and that have a p-value less than 0.05 or a q-value less than 0.10, or both a p-value less than 0.05 and a q-value less than 0.10, as determined by using a Welch's T-test or a Wilcoxon's rank sum Test. In other embodiments, the biomarkers that are administered are one or biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 that are decreased in a disease or condition and that have either a positive or negative correlation with a disease or condition. In one embodiment, the biomarkers have a positive or negative correlation either $\geq +0.5$ or ≤ -0.5, respectively, with a disease or condition. In other embodiments, the biomarkers that are administered are one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 that are decreased in a disease or condition by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, or by 100% (i.e., absent). In one example, the identification of biomarkers for insulin resistance also allows for the treatment of insulin resistance. For example, in order to treat a subject having insulin resistance, an effective amount of one or more insulin resistance biomarkers that are lowered in subjects having insulin resistance as compared to a healthy subject not having insulin resistance may be administered to the subject. The biomarkers that may be administered may comprise one or more of the biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28, and/or 29 that are decreased in a subject having insulin resistance. Although insulin resistance is discussed in this example, the other diseases or conditions, such as type-2 diabetes, metabolic syndrome, atherosclerosis, and cardiomyopathy, may also be treated in accordance with this method by using one or more of the respective biomarkers as set forth above.

X. Methods of Using the Biomarkers for Other Diseases or Conditions

[00148] In another aspect, at least some of the biomarkers disclosed herein for a particular disease or condition may also be biomarkers for other diseases or conditions. For example, it is believed that at least some of the insulin resistance biomarkers may be used in the methods described herein for other diseases or conditions (e.g., metabolic

syndrome). That is, the methods described herein with respect to insulin resistance may also be used for diagnosing (or aiding in the diagnosis of) a disease or condition such as type-2 diabetes, metabolic syndrome, atherosclerosis, or cardiomyopathy, methods of monitoring progression/regression of such a disease or condition, methods of assessing efficacy of compositions for treating such a disease or condition, methods of screening a composition for activity in modulating biomarkers associated with such a disease or condition, methods of identifying potential drug targets for such diseases and conditions, and methods of treating such diseases and conditions. Such methods could be conducted as described herein with respect to insulin resistance.

XI. Other methods

[00149] Other methods of using the biomarkers discussed herein are also contemplated. For example, the methods described in U.S. Patent No. 7,005,255 and U.S. Patent Application Nos. 11/357,732, 10/695,265 (Publication No. 2005/0014132), 11/301,077 (Publication No. 2006/0134676), 11/301,078 (Publication No. 2006/0134677), 11/301,079 (Publication No. 2006/0134678), and 11/405,033 may be conducted using a small molecule profile comprising one or more of the biomarkers disclosed herein.

[00150] In any of the methods listed herein, the biomarkers that are used may be selected from those biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 having p-values of less than 0.05 and/or those biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 having q-values less than 0.10, and/or having a positive or negative correlation either $\geq +0.5$ or ≤ -0.5, respectively, with a disorder. The biomarkers that are used in any of the methods described herein may also be selected from those biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 that are decreased in a metabolic syndrome-related metabolic disorder (as compared to the control or remission) or that are decreased in remission (as compared to control or a particular disease or condition) by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at

least 85%, by at least 90%, by at least 95%, or by 100% (i.e., absent); and/or those biomarkers in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and/or 29 that are increased in a given disease or condition (as compared to the control or remission) or that are increased in remission (as compared to the control or a given disease or condition) by at least 5%, by at least 10%, by at least 15%, by at least 20%, by at least 25%, by at least 30%, by at least 35%, by at least 40%, by at least 45%, by at least 50%, by at least 55%, by at least 60%, by at least 65%, by at least 70%, by at least 75%, by at least 80%, by at least 85%, by at least 90%, by at least 95%, by at least 100%, by at least 110%, by at least 120%, by at least 130%, by at least 140%, by at least 150%, or more.

Examples

I. General Methods

A. Identification of Metabolic Profiles

[00151] Each sample was analyzed to determine the concentration of several hundred metabolites. Analytical techniques such as GC-MS (gas chromatography-mass spectrometry) and LC-MS (liquid chromatography-mass spectrometry) were used to analyze the metabolites. Multiple aliquots were simultaneously, and in parallel, analyzed, and, after appropriate quality control (QC), the information derived from each analysis was recombined. Every sample was characterized according to several thousand characteristics, which ultimately amount to several hundred chemical species. The techniques used were able to identify novel and chemically unnamed compounds.

B. Statistical Analysis:

[00152] The data was analyzed using several statistical methods to identify molecules (either known, named metabolites or unnamed metabolites) present at differential levels in a definable population or subpopulation (e.g., biomarkers for metabolic syndrome biological samples compared to control biological samples or compared to patients in remission from insulin resistance) useful for distinguishing between the definable populations (e.g., insulin resistance and control, insulin resistance and remission, remission and control). Other molecules (either known, named metabolites or unnamed metabolites) in the definable population or subpopulation were also identified.

[00153] Random forest analyses were used for classification of samples into groups (e.g. disease or healthy, insulin resistant or normal insulin sensitivity, atherosclerosis or normal, metabolic syndrome or obese but not metabolic syndrome). Random forests give an estimate of how well we can classify individuals in a new data set into each group, in contrast to a t-test, which tests whether the unknown means for two populations are different or not. Random forests create a set of classification trees based on continual sampling of the experimental units and compounds. Then each observation is classified based on the majority votes from all the classification trees.

[00154] Regression analysis was performed using the Random Forest Regression method and the Univariate Correlation/Linear Regression method to build models that are useful to identify the biomarker compounds that are associated with disease or disease indicators (e.g. atherosclerosis, metabolic syndrome, Rd) and then to identify biomarker compounds useful to classify individuals according to for example, the level of glucose utilization as normal, insulin impaired, or insulin resistant. Biomarker compounds that are useful to predict disease or measures of disease (e.g. atherosclerosis, metabolic syndrome, Rd) and that are positively or negatively correlated with disease or measures of disease (e.g. atherosclerosis, metabolic syndrome, Rd) were identified in these analyses. All of the biomarker compounds identified in these analyses were statistically significant ($p<0.05$, $q<0.1$).

[00155] Recursive partitioning relates a 'dependent' variable (Y) to a collection of independent ('predictor') variables (X) in order to uncover -- or simply understand -- the elusive relationship, $Y=f(X)$. The analysis was performed with the JMP program (SAS) to generate a decision tree. The statistical significance of the "split" of the data can be placed on a more quantitative footing by computing p-values, which discern the quality of a split relative to a random event. The significance level of each "split" of data into the nodes or branches of the tree was computed as p-values, which discern the quality of the split relative to a random event. It was given as LogWorth, which is the negative log10 of a raw p-value.

[00156] Statistical analyses were performed with the program "R" available on the worldwide web at the website cran.r-project.org and in JMP 6.0.2 (SAS® Institute, Cary, NC).

Example 2: Biomarkers of Pre-Diabetes

2A: Identification of biomarkers that correlate with glucose disposal

[00157] A combination of biomarkers were discovered that, when used in an algorithm, correlate with the glucose disposal rate (i.e. Rd). Further, the initial panel of biomarkers can be narrowed for the development of targeted assays comprised of 15-30 candidate metabolites. An algorithm to predict insulin resistance was developed.

[00158] Several studies were conducted to identify biomarkers that correlate with glucose disposal. In a first study, plasma samples were collected from 113 lean, obese or diabetic subjects that had received treatment with one of three different thiazolidinedione drugs (T= troglitazone, R = rosiglitazone, or P = pioglitazone) (Table 1). Base line samples obtained from the subjects prior to treatment (S = baseline) served as controls. One to three plasma samples were obtained from each subject, with samples collected at baseline (all subjects; A), and after 12 weeks (B) or 4 weeks (C) of drug treatment (Table 2). Glucose disposal rate was measured in every subject by the hyperinsulinemic euglycemic (HI) clamp following each blood draw. A total of 198 plasma samples were collected for analysis.

Table 1. Gender and treatments of the study 1 cohort.

GROUP	GENDER	P	R	S	T	*Total*
Lean	F	1	0	1	1	*3*
	M	7	0	12	8	*27*
Obese	F	2	0	3	1	*6*
	M	7	0	14	8	*29*
Diabetic	F	0	7	3	1	*11*
	M	8	13	7	9	*37*
Total		***25***	***20***	***40***	***28***	***113***

Table 2. Treatment and collection time of the study 1 cohort.

GROUP	TIME	P	R	S	T	Total
L	A	8	0	13	9	30
	B	8	0	0	8	16
O	A	9	0	17	9	35
	B	9	0	0	9	18

	C	9	0	0	0	9
D	A	8	19	10	9	46
	B	8	20	0	10	38
	C	6	0	0	0	6
Total		*65*	*39*	*40*	*54*	*198*

[00159] In a second study, plasma samples were collected from 402 subjects that were balanced for age and gender. The subjects underwent HI clamp to determine the glucose disposal rate (Rd) of each individual. Based upon an Oral Glucose Tolerance Test (OGTT) or a Fasting Plasma Glucose Test (FPGT) the glucose tolerance of the subjects was designated as Normal glucose tolerance (NGT), Impaired Fasting Glucose (IFG) or Impaired Glucose Tolerance (IGT). The cohort is described in Table 3.

Table 3. Cohort Description, Study 2

			Age		Rd	
Group	Gender	N	Mean	Std Dev	Mean	Std Dev
NGT	female	155	44.64	8.02	8.5	3.09
	male	148	44.03	8.62	8.38	2.77
IFG	female	5	46.8	6.53	6.13	3.32
	male	12	45.25	9.63	4.67	2.57
IGT	female	45	45.56	7.81	4.19	1.81
	male	37	45.73	7.8	4.73	2.27

Abbreviations
Rd: Glucose disposal rate
NGT: Normal Glucose Tolerant (OGTT, <140 mg/dL or < 7.8 mmol/L)
IFG: Impaired Fasting Glucose (Fasting plasma glucose, 100-125 mg/dL or 5.6-6.9 mmol/L)
IGT: Impaired Glucose Tolerant (OGTT, 140-199 mg/dL or 7.8 – 11.0 mmol/L)

[00160] All samples from both studies were analyzed by GC-MS and LC-MS to identify and quantify the small molecules present in the samples. Over 400 compounds were detected in the samples.

[00161] Statistical analyses was performed to determine the compounds that are useful as biomarkers. Linear regression was used to correlate the baseline levels of individual compounds with the glucose disposal rate (Rd) as measured by the euglycemic hyperinsulinemic clamp for each individual. This analysis was followed by Random Forest analysis to identify variables most useful for Rd modeling. Then, LASSO regression analysis was performed on the cross-validated variables from the Random

Forest analysis to pick the combination of variables useful to predict Rd.

2B: Biomarkers of glucose utilization, molecules positively and negatively correlated with glucose disposal rate (Rd)

[00162] Biomarkers were discovered by (1) analyzing blood samples drawn from different groups of human subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the groups of subjects and the metabolites that correlate with the glucose disposal rate, an indicator of insulin sensitivity.

[00163] The plasma samples used for the analysis were from the cohorts described in Tables 1, 2 and 3; the subjects had various rates of glucose disposal (Rd). Based on the Rd value subjects were classified as insulin resistant ($Rd \leq 4$), insulin impaired ($4 < Rd < 7.5$) or insulin sensitive ($Rd > 7.5$). After the levels of metabolites were determined, the data was analyzed using Univariate Correlation/Linear Regression.

[00164] As listed below in Table 4, biomarkers were discovered that were correlated with the glucose disposal rate (Rd), an indicator of insulin sensitivity.

[00165] Table 4 includes, for each listed biomarker, the p-value determined in the statistical analysis of the data concerning the biomarkers, and the correlation (Corr) with Rd. A positive correlation indicates that the level of the biomarker increases as the glucose disposal rate increases. A negative correlation indicates that the level of the biomarker decreases as the glucose disposal rate increases. The range of possible correlations is between negative (-)1.0 and positive (+)1.0. A result of negative (-)1.0 means a perfect negative correlation, a positive (+)1.0 means a perfect positive correlation, and 0 means no correlation at all. The term "Isobar" as used in the table indicates the compounds that could not be distinguished from each other on the analytical platform used in the analysis (i.e., the compounds in an isobar elute at nearly the same time and have similar (and sometimes exactly the same) quant ions, and thus cannot be distinguished).

[00166] The results of this analysis showed the individual compounds that are correlated with Rd in both study 1 and study 2; these biomarkers are listed in Table 4. For each biomarker the study, compound name, database identifier, median importance are given. The Library ID (LIB_ID) indicates the analytical platform that was used to

measure the biomarker compound. GC-MS is indicated by Library ID (Lib_ID) 50 whereas LC-MS is indicated by Library ID 61, 200, and 201. The biomarker compounds are ordered in the table by the statistical significance of the correlation (P-value). "RF_Rank" refers to the importance score obtained for the biomarker from Random Forest analysis. "Comp_ID" refers to the internal database identifier for that compound in our internal compound library.

Table 4. Biomarker correlation with Rd

Study	Comp _ID	Compound Name	Lib _ID	RF_ Rank	Correlation	R-square	P-value
2	21044	2-hydroxybutyrate (AHB)	50	1	-4.47E-01	0.200	1.21E-14
1	20488	glucose	50	1	3.69E-01	0.136	1.21E-14
1	587	gluconate	50	2	-6.11E-01	0.373	8.73E-13
1	1336	palmitate (16:0)	201	5	-5.95E-01	0.355	4.37E-12
1	33416	Metabolite - 12064	201	9	-5.94E-01	0.353	5.09E-12
1	20675	1,5-anhydroglucitol (1,5-AG)	201	13	5.93E-01	0.352	5.62E-12
1	12751	glutamate-2	50	3	-5.82E-01	0.339	1.66E-11
1	1121	margarate (17:0)	50	4	-5.52E-01	0.304	2.88E-10
1	584	mannose	50	6	-5.50E-01	0.303	3.28E-10
1	31535	Bradykinin, hydroxyproline form	61	6	1.22E-08	0.295	7.09E-10
1	21044	2-hydroxybutyrate (AHB)	50	14	-5.40E-01	0.292	7.75E-10
1	27719	galactonic acid	50	1	-5.40E-01	0.291	8.28E-10
1	16235	Isobar.19 (1,5-AG etc)	61	31	1.96E-08	0.287	1.43E-09
1	33388	Metabolite - 12037	201	19	-5.32E-01	0.283	1.58E-09
2	1359	oleate (18:1(n-9))	201	3	-3.54E-01	0.125	2.15E-09
1	1358	stearate (18:0)	201	15	-5.22E-01	0.273	3.57E-09
1	27392	dipalmitin	50	8	-5.18E-01	0.268	4.97E-09
1	32569	Metabolite - 11252	200	56	-5.10E-01	0.260	9.09E-09
1	33232	Metabolite - 11887	201	41	-5.03E-01	0.253	1.60E-08
1	21047	3-methyl-2-oxobutyrate	201	42	-5.03E-01	0.253	1.64E-08
1	10737	Isobar.1 (mannose, glucose etc)	61	36	1.72E-07	0.254	1.75E-08
1	22570	Metabolite - 9033	50	62	-4.98E-01	0.248	2.24E-08
1	32566	Metabolite - 11249	200	70	-4.95E-01	0.245	2.82E-08
1	18369	gamma-glutamylleucine	200	12	-4.95E-01	0.245	2.95E-08
1	25602	Metabolite - 10432	50	39	-4.91E-01	0.241	3.79E-08
1	27722	erythrose	50	43	-4.91E-01	0.241	3.84E-08
1	32630	oleate (18:1(n-9))	201	54	-4.86E-01	0.236	5.47E-08
1	27888	Metabolite - 10609	50	10	-4.85E-01	0.235	5.87E-08
1	24077	Metabolite - 9727	50	22	-4.85E-01	0.235	6.15E-08
1	32696	Metabolite - 11379	201	37	-4.84E-01	0.234	6.50E-08
1	12666	Threonine	50	9	4.82E-07	0.235	7.02E-08
1	32551	Metabolite - 11234	201	34	-4.83E-01	0.233	7.11E-08
1	30288	Metabolite - 10750	50	25	-4.82E-01	0.232	7.39E-08
1	33413	Metabolite - 12061	201	16	-4.80E-01	0.231	8.27E-08

1	60	leucine	200	58	-4.80E-01	0.230	8.52E-08
1	21127	palmitoylglycerol (monopalmitin)	50	60	-4.75E-01	0.225	1.23E-07
1	32393	glutamylvaline	200	11	-4.74E-01	0.225	1.26E-07
1	19462	Metabolite - 6446	50	30	-4.74E-01	0.225	1.28E-07
1	16120	Metabolite - 4055	50	38	-4.73E-01	0.223	1.44E-07
1	32515	valine	200	71	-4.72E-01	0.223	1.49E-07
1	5628	Metabolite- 1086	61	5	1.12E-06	0.221	1.96E-07
1	32704	Metabolite - 11387	200	115	-4.68E-01	0.219	1.99E-07
1	32571	Metabolite - 11254	200	97	-4.66E-01	0.217	2.25E-07
1	32501	dihomo-alpha-linolenate (20:3(n-3))	201	26	-4.66E-01	0.217	2.31E-07
1	17627	Metabolite - 4986	50	24	-4.61E-01	0.213	3.05E-07
1	32402	gondoate (20:1(n-9))	201	101	-4.48E-01	0.201	7.10E-07
1	32575	Metabolite - 11258	200	241	-4.47E-01	0.200	7.75E-07
1	32970	Metabolite - 11653	201	51	-4.45E-01	0.198	8.81E-07
1	16512	Metabolite- 4275	50	54	3.95E-06	0.2	8.91E-07
1	12757	Metabolite - 3078	50	52	4.44E-01	0.197	9.64E-07
1	27718	creatine	200	27	-4.41E-01	0.194	1.16E-06
1	30832	Metabolite - 10814	50	47	-4.40E-01	0.193	1.23E-06
1	30290	Metabolite - 10752	50	121	-4.38E-01	0.192	1.36E-06
1	32703	Metabolite - 11386	200	94	-4.37E-01	0.191	1.47E-06
1	577	fructose	50	93	-4.36E-01	0.190	1.53E-06
1	22116	4-methyl-2-oxopentanoate	201	74	-4.35E-01	0.189	1.65E-06
1	33172	Metabolite - 11827	201	61	4.35E-01	0.189	1.67E-06
1	22600	Metabolite - 9043	50	90	-4.31E-01	0.186	2.05E-06
1	1125	isoleucine	200	137	-4.31E-01	0.185	2.14E-06
1	19490	Metabolite - 6488	50	32	-4.28E-01	0.183	2.46E-06
1	16518	Metabolite - 4276	50	18	-4.26E-01	0.182	2.78E-06
1	15122	glycerol	50	45	-4.25E-01	0.181	2.93E-06
1	12782	Metabolite - 3100	50	17	-4.23E-01	0.179	3.39E-06
1	33242	Metabolite - 11897	201	77	-4.21E-01	0.177	3.75E-06
1	17330	Metabolite - 4769	50	35	4.21E-01	0.177	3.86E-06
1	32672	Metabolite - 02546_200	200	67	4.14E-01	0.171	5.69E-06
1	33237	Metabolite - 11892	201	124	-4.12E-01	0.170	6.48E-06
1	32673	linoleate (18:2(n-6))	201	28	-4.07E-01	0.166	8.49E-06
1	32545	Metabolite - 11228	201	64	-4.03E-01	0.163	1.05E-05
1	32751	Metabolite - 11434	201	63	-4.03E-01	0.162	1.06E-05
1	22895	Metabolite - 9299	50	72	-3.99E-01	0.159	1.34E-05
2	33488	5-alpha-Cholest-7-en-3-beta-ol	50	11	-2.61E-01	0.068	1.40E-05
1	32517	Metabolite - 11203	200	82	3.98E-01	0.158	1.42E-05
1	33415	Metabolite - 12063	201	44	-3.97E-01	0.158	1.46E-05
1	32682	Metabolite - 11365	201	29	-3.94E-01	0.155	1.72E-05
2	32761	Metabolite - 11444	201	12	-2.58E-01	0.067	1.77E-05
2	32338	glycine	50	13	2.58E-01	0.066	1.83E-05
1	32749	Metabolite - 11432	201	107	-3.91E-01	0.153	2.04E-05
1	1110	arachidonate (20:4(n-6))	50	49	-3.90E-01	0.152	2.12E-05
1	33138	Metabolite - 11793	200	96	-3.88E-01	0.150	2.39E-05
2	33447	palmitoleate (16:1(n-7))	201	15	-2.53E-01	0.064	2.59E-05
1	32504	n-3 DPA (22:5(n-3))	201	31	-3.83E-01	0.147	3.05E-05
1	18868	Metabolite - 5847	50	80	-3.80E-01	0.144	3.56E-05
1	27738	threonate	50	53	3.75E-01	0.141	4.62E-05

1	32552	Metabolite - 11235	201	73	-3.75E-01	0.140	4.68E-05
1	27279	Metabolite - 10511	50	7	-3.69E-01	0.136	6.11E-05
1	32547	Metabolite - 11230	201	163	-3.68E-01	0.135	6.55E-05
1	19377	Metabolite - 6272	50	194	-3.66E-01	0.134	7.40E-05
1	32416	alpha-linolenate (18:3(n-3))	201	65	-3.66E-01	0.134	7.41E-05
1	33080	Metabolite - 11735	200	50	3.63E-01	0.132	8.27E-05
1	22320	Metabolite - 8889	50	177	3.62E-01	0.131	8.90E-05
1	32945	Metabolite - 11628	201	197	-3.60E-01	0.130	9.48E-05
2	599	pyruvate	50	18	-2.31E-01	0.053	1.00E-04
2	33453	alpha-ketoglutarate	50	17	-2.35E-01	0.055	1.00E-04
2	1105	linoleate (18:2(n-6))	201	16	-2.44E-01	0.059	1.00E-04
1	527	lactate	50	76	-3.58E-01	0.128	1.06E-04
1	15676	3-methyl-2-oxovalerate	201	216	-3.53E-01	0.125	1.35E-04
1	32836	peptide - HWESASXX	200	33	-3.47E-01	0.121	1.76E-04
1	32637	Metabolite - 11320	201	100	3.45E-01	0.119	1.91E-04
2	15749	hydrocinnamic acid	201	21	2.26E-01	0.051	2.00E-04
2	1648	serine	50	19	2.27E-01	0.052	2.00E-04
1	15500	carnitine	200	310	-3.38E-01	0.114	2.64E-04
1	16496	pyruvate	50	119	-3.35E-01	0.113	3.00E-04
1	32559	Metabolite - 11242	201	87	-3.35E-01	0.112	3.11E-04
1	32632	Metabolite - 11315	200	110	3.31E-01	0.110	3.61E-04
2	33587	Isobar-cis-9-cis-11-trans-11-eicosenoate	201	23	-2.16E-01	0.046	4.00E-04
2	32401	trigonelline (N-methylnicotinate)	200	22	2.16E-01	0.047	4.00E-04
1	21630	Metabolite - 8402	50	36	-3.27E-01	0.107	4.40E-04
1	16666	Metabolite - 4365	50	272	-3.25E-01	0.105	4.79E-04
1	16665	Metabolite - 4364	50	105	3.24E-01	0.105	4.98E-04
1	19983	Metabolite - 6955	50	122	-3.24E-01	0.105	4.99E-04
2	32405	3-indolepropionate	50	24	2.10E-01	0.044	5.00E-04
1	31509	Metabolite - 10931	50	220	-3.21E-01	0.103	5.66E-04
1	27889	Metabolite - 10610	50	95	-3.19E-01	0.102	6.12E-04
1	16650	Metabolite - 4360	50	125	-3.18E-01	0.101	6.37E-04
1	32644	Metabolite - 11327	200	111	-3.18E-01	0.101	6.39E-04
2	32445	3-methylxanthine	201	26	2.06E-01	0.042	7.00E-04
1	19370	Metabolite - 6268	50	179	3.14E-01	0.099	7.47E-04
1	32702	Metabolite - 11385	200	221	-3.13E-01	0.098	7.73E-04
1	19576	Metabolite - 6627	50	135	-3.12E-01	0.098	7.97E-04
2	32757	Metabolite - 11440	201	28	-2.03E-01	0.041	8.00E-04
1	12663	serine- 2	50	20	3.11E-01	0.097	8.44E-04
1	19494	Metabolite - 6506	50	236	-3.10E-01	0.096	8.82E-04
1	32628	palmitoleate (16:1(n-7))	201	83	-3.10E-01	0.096	8.90E-04
1	59	histidine	201	355	-3.09E-01	0.096	9.00E-04
1	32516	Metabolite - 11202	200	134	3.08E-01	0.095	9.69E-04
1	33087	peptide - RPPGFSPF	200	127	-3.04E-01	0.093	1.11E-03
2	31453	cysteine	50	29	-1.95E-01	0.038	1.30E-03
1	16138	Metabolite - 4080	50	371	-3.00E-01	0.090	1.30E-03
2	24074	Metabolite - 9706	50	30	1.94E-01	0.038	1.40E-03
1	32595	Metabolite - 08993_200	200	48	-2.97E-01	0.088	1.46E-03
1	16509	Metabolite - 4273	50	369	2.96E-01	0.088	1.50E-03
1	32735	Metabolite - 01911_200	200	146	-2.96E-01	0.088	1.53E-03
1	30281	glycine-2	50	154	2.95E-01	0.087	1.58E-03

1	32519	Metabolite - 11205	200	120	2.95E-01	0.087	1.59E-03
2	33531	Metabolite - 12116	200	31	1.91E-01	0.036	1.60E-03
1	64	phenylalanine	200	21	-2.90E-01	0.084	1.89E-03
1	32548	Metabolite - 11231	201	131	-2.90E-01	0.084	1.89E-03
1	22154	bradykinin	200	55	-2.89E-01	0.084	2.00E-03
1	32348	2-aminobutyrate	200	297	-2.86E-01	0.082	2.25E-03
1	31537	peptide - HWESASXXR	200	99	-2.84E-01	0.081	2.41E-03
1	32747	Metabolite - 01142_201	201	148	-2.83E-01	0.080	2.48E-03
1	32550	Metabolite - 02272_201	201	176	2.82E-01	0.080	2.59E-03
2	15753	hippurate	200	35	1.82E-01	0.033	2.70E-03
2	32198	acetylcarnitine	200	34	-1.82E-01	0.033	2.70E-03
1	21188	stearoylglycerol (monostearin)	50	196	-2.78E-01	0.077	2.97E-03
1	12626	Metabolite - 3003	50	198	2.77E-01	0.077	3.10E-03
1	32654	Metabolite - 11337	200	84	-2.73E-01	0.075	3.53E-03
1	21421	Metabolite - 8214	50	98	-2.73E-01	0.075	3.58E-03
2	32807	Metabolite - 11490	201	38	-1.74E-01	0.030	4.10E-03
1	606	uridine	201	230	-2.69E-01	0.072	4.16E-03
1	19487	Metabolite - 6486	50	78	-2.69E-01	0.072	4.18E-03
1	32412	butyrylcarnitine	200	389	-2.69E-01	0.072	4.20E-03
2	32616	Metabolite - 11299	201	41	1.72E-01	0.030	4.60E-03
1	25459	Metabolite - 10395	50	285	-2.62E-01	0.068	5.35E-03
1	33210	Metabolite - 11865	201	171	2.61E-01	0.068	5.47E-03
1	27264	Metabolite - 10503	50	181	2.59E-01	0.067	5.85E-03
1	32578	Metabolite - 11261	200	223	-2.58E-01	0.067	5.93E-03
1	32609	Metabolite - 01345_201	201	57	2.58E-01	0.067	5.94E-03
1	25609	Metabolite - 10439	50	150	-2.57E-01	0.066	6.19E-03
1	12768	Metabolite - 3088	50	91	2.56E-01	0.065	6.49E-03
1	18120	Metabolite - 5348	50	108	-2.56E-01	0.065	6.53E-03
2	3147	xanthine	50	44	-1.65E-01	0.027	6.60E-03
2	15990	glycerophosphorylcholine (GPC)	200	45	1.64E-01	0.027	6.90E-03
1	2730	gamma-glutamylglutamine	200	109	2.51E-01	0.063	7.60E-03
1	32701	urate	200	85	-2.50E-01	0.063	7.80E-03
1	33227	Metabolite - 11882	201	23	-2.49E-01	0.062	8.01E-03
1	19934	inositol	50	227	-2.44E-01	0.059	9.62E-03
1	25402	Metabolite - 10360	50	129	-2.44E-01	0.059	9.63E-03
1	32520	Metabolite - 11206	200	218	-2.43E-01	0.059	9.91E-03
2	32877	Metabolite - 11560	201	49	1.56E-01	0.024	1.02E-02
1	32753	Metabolite - 09789_201	201	193	2.42E-01	0.058	1.03E-02
2	1494	5-oxoproline	200	50	1.56E-01	0.024	1.04E-02
1	12774	Metabolite - 3094	50	199	-2.41E-01	0.058	1.05E-02
1	32635	Metabolite - 11318	201	175	2.41E-01	0.058	1.05E-02
1	20950	Metabolite - 7846	50	162	-2.40E-01	0.058	1.07E-02
1	32606	bilirubin	201	289	2.40E-01	0.058	1.08E-02
1	32752	Metabolite - 11435	201	184	-2.38E-01	0.057	1.16E-02
1	32754	Metabolite - 11437	201	186	2.34E-01	0.055	1.29E-02
1	12129	beta-hydroxyisovalerate	50	140	-2.34E-01	0.055	1.30E-02
1	17028	Metabolite - 4611	50	130	-2.34E-01	0.055	1.31E-02
1	33132	Metabolite - 11787	200	251	-2.34E-01	0.055	1.31E-02
1	12067	undecanoate	201	183	-2.33E-01	0.054	1.34E-02
1	542	3-hydroxybutyrate (BHBA)	50	206	-2.33E-01	0.054	1.35E-02

2	33323	Metabolite - 11977	200	51	-1.49E-01	0.022	1.41E-02
1	512	asparagine	50	75	2.31E-01	0.053	1.44E-02
2	54	tryptophan	200	52	1.47E-01	0.022	1.53E-02
1	22032	Metabolite - 8766	50	208	-2.28E-01	0.052	1.57E-02
2	32792	Metabolite - 11475	201	53	-1.46E-01	0.021	1.64E-02
1	32625	Metabolite - 11308	201	301	-2.26E-01	0.051	1.65E-02
1	32813	Metabolite - 11496	201	116	-2.25E-01	0.051	1.69E-02
1	18477	glycodeoxycholate	201	192	-2.24E-01	0.050	1.76E-02
2	12795	Metabolite - 3113	50	54	1.44E-01	0.021	1.78E-02
1	32553	Metabolite - 03832_201	201	256	-2.23E-01	0.050	1.82E-02
2	33364	gamma-glutamylthreonine-	200	56	1.43E-01	0.020	1.88E-02
2	2342	serotonin (5HT)	200	57	1.40E-01	0.020	2.12E-02
1	32855	Metabolite - 11538	201	46	2.17E-01	0.047	2.13E-02
1	32197	3-(4-hydroxyphenyl)lactate	201	190	-2.14E-01	0.046	2.38E-02
2	2132	citrulline	200	58	1.37E-01	0.019	2.41E-02
1	21049	1,6-anhydroglucose	50	136	2.13E-01	0.045	2.43E-02
1	33362	gamma-glutamylphenylalanine	200	173	-2.12E-01	0.045	2.49E-02
1	32452	propionylcarnitine	200	252	-2.12E-01	0.045	2.51E-02
1	32656	Metabolite - 11339	201	142	2.11E-01	0.045	2.55E-02
2	1365	myristate (14:0)	201	59	-1.36E-01	0.018	2.59E-02
1	25532	Metabolite - 10413	50	226	-2.10E-01	0.044	2.63E-02
2	3141	betaine	200	60	1.35E-01	0.018	2.64E-02
1	32648	Metabolite - 11331	201	132	2.10E-01	0.044	2.66E-02
1	25548	Metabolite - 10419	50	128	2.08E-01	0.043	2.76E-02
1	32748	Metabolite - 11431	201	178	-2.08E-01	0.043	2.79E-02
1	33135	Metabolite - 11790	200	138	-2.07E-01	0.043	2.84E-02
1	31518	Metabolite - 10933	50	152	-2.07E-01	0.043	2.85E-02
1	19478	Metabolite - 6467	50	118	-2.06E-01	0.042	2.95E-02
2	33226	Metabolite - 11881	201	65	1.32E-01	0.018	2.97E-02
1	32561	Metabolite - 11244	201	243	-2.05E-01	0.042	2.98E-02
1	11438	phosphate	50	273	-2.05E-01	0.042	3.02E-02
2	1572	glycerate	50	66	1.31E-01	0.017	3.10E-02
2	33477	erythronate-	50	67	1.31E-01	0.017	3.13E-02
1	12781	Metabolite - 3099	50	141	-2.03E-01	0.041	3.18E-02
1	32732	Metabolite - 11415	201	155	2.03E-01	0.041	3.22E-02
1	1299	tyrosine	200	147	-2.02E-01	0.041	3.31E-02
2	27256	Metabolite - 10500	50	69	-1.29E-01	0.017	3.37E-02
1	32346	glycochenodeoxycholate	201	202	-2.00E-01	0.040	3.45E-02
1	27710	N-acetylglycine	50	126	2.00E-01	0.040	3.47E-02
1	22842	cholate	201	165	-1.99E-01	0.040	3.56E-02
1	31373	Metabolite - 10878	50	274	1.98E-01	0.039	3.59E-02
2	16511	Metabolite - 4274	50	72	1.27E-01	0.016	3.71E-02
2	15996	aspartate	50	73	1.27E-01	0.016	3.72E-02
1	33228	Metabolite - 11883	200	332	1.97E-01	0.039	3.72E-02
1	18929	Metabolite - 5907	50	172	-1.96E-01	0.039	3.80E-02
2	569	caffeine	200	74	-1.26E-01	0.016	3.87E-02
2	32971	Metabolite - 11654	200	76	-1.25E-01	0.016	3.98E-02
1	32795	Metabolite - 11478	201	189	-1.94E-01	0.038	4.00E-02
1	32868	glycocholate	201	225	-1.93E-01	0.037	4.12E-02
2	18335	quinate	50	78	1.24E-01	0.015	4.24E-02
1	32587	Metabolite - 02249_201	201	89	-1.91E-01	0.036	4.38E-02

1	22548	Metabolite - 9026	50	337	-1.91E-01	0.036	4.39E-02
1	32829	Metabolite - 03653_200	200	102	1.90E-01	0.036	4.48E-02
1	33185	Metabolite - 11840	201	104	-1.90E-01	0.036	4.49E-02
1	20699	erythritol	50	133	-1.90E-01	0.036	4.50E-02
1	32588	Metabolite - 01327_201	201	233	-1.90E-01	0.036	4.52E-02
1	32848	Metabolite - 11531	201	245	-1.89E-01	0.036	4.54E-02
1	17389	Metabolite - 4796	50	79	1.89E-01	0.036	4.57E-02
2	1493	ornithine	50	82	1.22E-01	0.015	4.60E-02
2	32418	myristoleate*14-1-n-5-	201	84	-1.21E-01	0.015	4.68E-02
2	15140	kynurenine	200	83	1.21E-01	0.015	4.68E-02
1	32596	Metabolite - 02250_200	200	151	-1.88E-01	0.035	4.69E-02
1	33380	Metabolite - 12029	201	68	1.87E-01	0.035	4.78E-02
1	27272	Metabolite - 10505	50	393	1.86E-01	0.035	4.96E-02
1	63	cholesterol	50	212	-1.86E-01	0.035	4.96E-02
1	33198	Metabolite - 11853	201	314	-1.84E-01	0.034	5.21E-02
2	15630	N-acetylornithine	200	87	1.17E-01	0.014	5.52E-02
2	19323	docosahexaenoate (DHA)22-6-n-3-	201	90	-1.16E-01	0.013	5.66E-02
2	32760	Metabolite - 11443	201	91	-1.16E-01	0.013	5.68E-02
2	18392	theobromine	200	92	1.16E-01	0.013	5.73E-02
1	32514	Metabolite - 11200	200	201	1.79E-01	0.032	5.88E-02
2	16634	Metabolite - 4357	50	96	-1.13E-01	0.013	6.37E-02
1	32518	Metabolite - 11204	200	396	-1.75E-01	0.031	6.46E-02
1	33081	Metabolite - 11736	200	276	-1.75E-01	0.031	6.47E-02
1	32850	Metabolite - 11533	201	217	-1.73E-01	0.030	6.82E-02
1	32671	Metabolite - 11354	200	139	1.73E-01	0.030	6.86E-02
2	32786	Metabolite - 11469	200	98	1.11E-01	0.012	6.92E-02
2	25522	Metabolite - 10407	50	99	-1.10E-01	0.012	7.11E-02
2	1649	valine	200	100	-1.10E-01	0.012	7.21E-02
1	32684	Metabolite - 11367	201	224	-1.70E-01	0.029	7.23E-02
1	553	cotinine	200	266	1.70E-01	0.029	7.24E-02
1	33389	Metabolite - 12038	201	214	-1.70E-01	0.029	7.24E-02
1	1563	chenodeoxycholate	201	370	-1.70E-01	0.029	7.39E-02
2	22481	Metabolite - 8988	50	102	1.09E-01	0.012	7.48E-02
1	12593	Metabolite - 2973	50	149	1.67E-01	0.028	7.76E-02
1	32793	Metabolite - 11476	200	383	-1.67E-01	0.028	7.80E-02
1	32564	Metabolite - 11247	201	249	1.67E-01	0.028	7.84E-02
2	1508	pantothenate	200	104	-1.07E-01	0.012	7.84E-02
2	16829	Metabolite - 4503	50	105	1.07E-01	0.011	7.99E-02
1	32652	Metabolite - 11335	200	388	1.66E-01	0.028	8.03E-02
1	15365	glycerol 3-phosphate (G3P)	50	348	-1.65E-01	0.027	8.15E-02
2	32875	Metabolite - 11558	200	107	-1.06E-01	0.011	8.29E-02
2	15506	choline	200	108	1.06E-01	0.011	8.32E-02
2	32492	caprylate-8-0-	201	109	1.05E-01	0.011	8.51E-02
1	16287	Metabolite - 2800	50	209	-1.63E-01	0.027	8.51E-02
1	1114	deoxycholate	201	106	-1.63E-01	0.027	8.63E-02
2	32619	Metabolite - 11302	201	110	1.05E-01	0.011	8.64E-02
1	27275	Metabolite - 10507	50	182	1.62E-01	0.026	8.74E-02
1	32718	Metabolite - 01342_200	200	167	-1.61E-01	0.026	8.94E-02
2	33403	Metabolite - 12051	200	112	-1.03E-01	0.011	8.96E-02
1	32769	Metabolite - 11452	201	278	-1.59E-01	0.025	9.31E-02
2	32756	Metabolite - 02276_201	201	116	1.01E-01	0.010	9.72E-02

2	33225	Metabolite - 11880	201	118	-1.01E-01	0.010	9.88E-02
1	32839	Metabolite - 11522	201	144	1.57E-01	0.025	9.89E-02

2C: Variable Selection with Random Forest for Rd modeling

[00167] 50 iterations of a random forest analysis with complete 5-fold cross-validation regressions (for Study 1 this analysis included only baseline data, n=111; while for Study 2 all samples were included, n=402) was carried out as follows:

[00168] 80% of the data was used as the training set to run 1000 regression random forests, record the importance scores and rank the variables according to their importance scores;

[00169] Next, four variables at a time were deleted starting from the lowest ranked variables, then the random forest was run with the remaining variables on the training set to predict the remaining 20% of the data (i.e., test set). The error and R-square for each was recorded.

[00170] For each variable, the mean/median importance score and rank across all run was calculated.

[00171] Variable selection is more or less stable for the approximately first 30-60 variables.

2D: Estimate of the number of metabolites considered significant for Rd correlation

[00172] The mean R-square values remain constantly high and the corresponding errors remain consistently low as the number of metabolites reaches approximately 30 or more (Figures 1 and 2), suggesting that a total of approximately 30 metabolites may be sufficient for construction of an algorithm to correlate with Rd, although it may also be possible to construct an algorithm to correlate with Rd based on a combination of less than seven metabolites. As a result, only the top 30 to 50 cross-validated compounds were selected for regression analyses.

[00173] Based on random forest variable selection procedures, the biomarker compounds that are considered reliably significant for construction of an algorithm for Rd correlation were identified. The RF score for each of the biomarker compounds is listed in the column headed "RF_Rank" in Table 4.

2E: Modeling of Rd correlation with top compounds

[00174] Based on the modeling experiments, the mean R-square values remain constantly high and the corresponding errors remain consistently low as the number of metabolites reaches seven and above (Figures 3 and 4), suggesting that a combination of seven metabolites will be sufficient for construction of an algorithm to correlate with Rd, although it may also be possible to construct an algorithm to correlate with Rd based on a combination of less than seven metabolites.

2F: LASSO Regression

[00175] Only cross-validated variables from the random forest analyses above were used for LASSO regression to pick the best combination of variables to predict Rd. The most appropriate transformation of the cross-validated variables was also considered for the LASSO regression.

[00176] LASSO regression analysis based upon the cohort in study 1 provided one of the best models of Rd regression with three to nine variables and cross-validated r-square values for the correlation. The best r-square value approaches 0.68 with seven to eight metabolites using the non-transformed data (Table 5) and approaches 0.69-0.70 with the same number of metabolites with appropriate transformation of each variable (Table 6).

Table 5. LASSO regression with non-transformed data.

Number of Variables	3	4	5	6	7	8	9
LASSO Maximum R-square	0.355	0.422	0.600	0.643	0.670	0.720	0.779
Cross-Validated R-square	0.586	0.600	0.653	0.651	0.681	0.687	0.665
1,5-Anhydro-D-glucitol	√	√	√	√	√	√	√
Bradykinin-hydroxyproline	√	√	√	√	√	√	√
Palmitate	√	√	√	√	√	√	√
Metabolite-9727			√	√	√	√	√
Glu-Val			√	√	√	√	√
Threonine		√			√	√	√
Dihydroimidazole-4-acetate					√	√	√
Mannose						√	

2-Hydroxybutyrate				√			
Isobar-56**							√
Serine							√

Note: ** Isobar 56 includes DL-pipecolic acid and 1-amino-1-cyclopentanecarboxylic acid that can be separated

Table 6. LASSO regression with transformed data.

Number of Variables	3	4	5	6	7	8	9
LASSO Maximum R-square	0.355	0.422	0.600	0.643	0.670	0.720	0.779
Cross-Validated R-square	0.592	0.603	0.662	0.657	0.692	0.702	0.684
1,5-Anhydro-D-glucitol	√	√	√	√	√	√	√
Bradykinin-hydroxyproline form	√	√	√	√	√	√	√
Log(Palmitate)	√	√	√	√	√	√	√
Log(Metabolite-9727)			√	√	√	√	√
Glu-Val			√	√	√	√	√
Threonine		√			√	√	√
Log(Dihydroimidazole-4-acetate)					√	√	√
Log(Mannose)						√	
Log(2-Hydroxybutyrate)				√			
Isobar-56**							√
Serine							√

Note: ** Isobar 56 includes DL-pipecolic acid and 1-amino-1-cyclopentanecarboxylic

[00177] The R-square for the correlation of Rd with 7-8 metabolites approaches 0.70 with cross-validation in an independent cohort.

[00178] LASSO analysis based on the cohort in study 1 provided the best models of Rd regression with 3-9 variables with cross-validated r-square values for the correlation of another set approaching 0.68 with 7-8 metabolites using the non-transformed data as shown in Table 7.

Table 7. LASSO regression with non-transformed data.

Number of Variables	3	4	5	6	7	8	9

Cross-Validated R-square	0.617	0.636	0.650	0.656	0.678	0.681	0.685
1,5-Anhydro-D-glucitol	√	√	√	√	√	√	√
Palmitate	√	√	√	√	√	√	√
Glu-Val	√	√	√	√	√	√	√
Serine		√	√	√	√	√	√
Margarate			√	√	√	√	√
X-9727				√	√	√	√
X-10511					√	√	
Etio cholanolone sulfate (X-1345)						√	√
Gamma tocopherol (X-4276)							√
Creatine							√

2G: Models predictive of insulin resistance.

[00179] In study 2, compounds identified as important in building models to predict Rd by Random Forest and Lasso Regression are listed in Table 4. The cross-validated compounds were then selected for regression analysis along with clinical measurements (e.g. fasting insulin, fasting pro-insulin, fasting free fatty acids (FFA), fasting C-peptide, HDL cholesterol, LDL cholesterol, fasting plasma glucose, adiponectin, BMI, PYY, etc.) Each regression method and the Univariate Correlation/Linear Regression method model was then used to predict Rd for each individual, which was in turn used to classify individuals according to the level of glucose utilization as normal, insulin impaired, or insulin resistant. Samples from ninety percent of the subjects were used to build the model and samples from the remaining ten percent of the subjects were used to test the predictive power of the model. Biomarker compounds that are useful to predict Rd and that are positively or negatively correlated with Rd were identified in these analyses. These markers are useful to predict insulin resistance. All of the biomarker compounds are statistically significant ($p<0.05$) in each of the regression models.

[00180] The models generated using this analytical approach are summarized in Table 8. The sensitivity, specificity and predictive power (positive, PPV and negative, NPV) of the models are shown in Table 8. The sensitivity of the models ranges from about 54% to about 63% and the specificity ranges from about 63% to greater than 95%. The PPV range is from about 78% to about 94% and the NPV from greater than about 79% to

greater than about 83%.

Table 8. Metabolite Biomarkers and models that are predictive of Insulin Resistance as determined by glucose disposal rate (Rd).

Model No.	No.Variables	R-square	Sensitivity	Specificity	PPV	NPV	Variable 1	Variable 2	Variable 3	Variable 4	Variable 5	Variable 6	Variable 7	Variable 8	Variable 9
1	8	0.5486	59.84	95.51	87.36	82.11	BMI	Fasting Insulin	Fasting_ Proinsulin	Fasting FFA	Adiponectin	GlulVal	Betaine	2Hydroxybutyrate	
2	9	0.4937	55.12	95.51	86.42	80.41	Fasting Insulin	Oleate	BMI	LDL_Cholesterol	FPG	Linoleate	2Hydroxybutyrate	Linolenate	HDL Cholesterol
3	7	0.5398	59.06	95.10	86.21	81.75	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	GlulVal	2Hydroxybutyrate		
4	9	0.5137	56.69	95.10	85.71	80.90	BMI	Fasting Insulin	2Hydroxybutyrate	Glul.Val	Creatine	Pyruvate	Gluconate	Glycine	Linolenate
5	9	0.5308	60.63	95.10	86.52	82.33	Fasting Insulin	Fasting Proinsulin	Glul.Val	BMI	Betaine	2Hydroxybutyrate	Oleate	Adiponectin	Gluconate
6	7	0.5122	56.69	94.69	84.71	80.84	BMI	Fasting Insulin	Pyruvate	Betaine	.Gamma.Glu. Leu	2Hydroxybutyrate	Gluconate		
7	8	0.5179	56.69	94.69	84.71	80.84	BMI	Fasting Insulin	Pyruvate	.Gamma.Glu. Leu	2Hydroxybutyrate	.Linolyl.LPC	Creatine	Gluconate	
8	9	0.5179	56.69	94.69	84.71	80.84	BMI	Fasting Insulin	Lactate	Pyruvate	Betaine	.Galactonate	2Hydroxybutyrate	.Linolyl.LPC	Gluconate
9	7	0.5380	62.20	94.69	85.87	82.86	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	HDL Cholesterol	.Gamma.Glu. Leu	2Hydroxybutyrate		
10	7	0.5458	61.42	94.69	85.71	82.56	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	2Hydroxybutyrate		
11	8	0.5531	60.63	94.69	85.56	82.27	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	2Hydroxybutyrate	Creatine	
12	8	0.5534	59.84	94.69	85.39	81.98	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Betaine	.Gamma.Glu. Leu	2Hydroxybutyrate	
13	9	0.5596	62.99	94.69	86.02	83.15	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Betaine	.Gamma.Glu. Leu	2Hydroxybutyrate	Gluconate
14	9	0.5584	59.84	94.69	85.39	81.98	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu.	2Hydroxybut	Creatine	Gluconate

15	9	0.5580	59.84	94.69	85.39	81.98	BMI	Fasting Insulin	Fasting Proinsulin	.LDL_Cholesterol	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	.Linoleate	2Hydroxybutyrate
16	9	0.5223	56.69	94.69	84.71	80.84	BMI	Fasting Insulin	2Hydroxybutyrate	Gamma.Glu.Leu	lin_Linolyl.LPC	Betaine	Pyruvate	Gluconate	X9033
17	9	0.5317	60.63	94.69	85.56	82.27	Fasting Insulin	Glul.Val	BMI	Betaine	Fasting Proinsulin	2Hydroxybutyrate	Linolyl.LPC	Adiponectin	Gluconate
18	9	0.5106	57.48	94.29	93.91	81.05	BMI	Fasting Insulin	2Hydroxybutyrate	Pyruvate	Oleoyl.LPC	Gluconate	Oleate	Galactonate	Linoleate
19	9	0.5167	55.91	94.29	83.53	80.49	BMI	Fasting Insulin	2Hydroxybutyrate	Betaine	Glul.Val	Creatine	Gluconate	lin_Glycine	X9033
20	9	0.5368	61.42	94.29	84.78	82.50	Fasting Insulin	Fasting Proinsulin	BMI	Betaine	Gamma.Glu.Leu	2Hydroxybutyrate	Oleate	Adiponectin	Gluconate
21	9	0.5253	59.84	94.29	84.44	81.91	Creatine	Glycine	Fasting Insulin	Fasting Proinsulin	Glul.Val	BMI	2Hydroxybutyrate	Adiponectin	Gluconate
22	9	0.5260	58.27	94.29	84.09	81.34	Fasting Insulin	Fasting Proinsulin	Pyruvate	BMI	FPG	2Hydroxybutyrate	Lactate	Adiponectin	Gluconate
23	9	0.4943	56.69	94.29	83.72	80.77	Fasting Insulin	BMI	FPG	Linoleate	2Hydroxybutyrate	Linolenate	HDL Cholesterol	Hepadecenate	Oleate
24	9	0.4864	55.12	94.29	83.33	80.21	Fasting Insulin	BMI	FPG	Galactonate	1.5.Anhydroglucitol	2Hydroxybutyrate	Lactate	Oleate	HDL Cholesterol
25	9	0.4958	54.33	94.29	83.13	79.93	Triglycerides	Linolenate	Fasting Insulin	Lactate	BMI	LDL_Cholesterol	FPG	1.5.Anhydroglucitol	2Hydroxybutyrate
26	7	0.5401	57.48	94.29	83.91	81.05	BMI	Fasting Insulin	Fasting FFA	Adiponectin	Gamma.Glu.Leu	2Hydroxybutyrate	Creatine		
27	8	0.5499	59.06	94.29	84.27	81.63	BMI	Fasting Insulin	Fasting Proinsulin	.FPG	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	2Hydroxybutyrate	
28	8	0.5492	59.06	94.29	84.27	81.63	BMI	Fasting Insulin	Fasting Proinsulin	.LDL_Cholesterol	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	2Hydroxybutyrate	
29	9	0.5578	62.99	94.29	85.11	83.09	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	.Linoleate	2Hydroxybutyrate	Linolenate
30	9	0.5576	62.99	94.29	85.11	83.09	BMI	Fasting Insulin	Fasting Proinsulin	.FPG	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	.Linoleate	2Hydroxybutyrate

31	9	0.5615	59.84	94.29	84.44	81.91	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	Gamma.Glu. Leu	.Linoleate	2Hydroxybut yrate	Creatine
32	9	0.4972	60.63	93.88	83.70	82.14	BMI	Fasting Insulin	2Hydroxybut yrate	Gluconate	Glycine	Lactate	Galactonate	Threonine	X1.5.Anhydr oglucitol
33	9	0.5060	55.91	93.88	82.56	80.42	BMI	Fasting Insulin	2Hydroxybut yrate	Glutamate	Pyruvate	Oleoyl.LPC	Gluconate	Glycine	Galactonate
34	9	0.5635	62.99	93.88	84.21	83.03	Fasting Insulin	Fasting Proinsulin	Gamma.Glu. Leu	Linoleate	BMI	Fasting FFA	2Hydroxybut yrate	Adiponectin	Gluconate
35	9	0.4996	55.12	93.88	82.35	80.14	Fasting Insulin	Fasting_C_Pep tide	Lactate	BMI	LDL_Cholest erol	FPG	Glycine	Triglycerides	2Hydroxybut yrate
36	9	0.4983	54.33	93.88	82.14	79.86	Fasting Insulin	Lactate	BMI	LDL_Cholest erol	FPG	Glycine	Triglycerides	1.5.Anhydrog lucitol	2Hydroxybut yrate
37	7	0.5136	55.12	93.88	82.35	80.14	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	Creatine	Gluconate		
38	8	0.5177	55.91	93.88	82.56	80.42	BMI	Fasting Insulin	Pyruvate	.Betaine	.Galactonate	2Hydroxybut yrate	.Linolyl.LPC	Gluconate	
39	8	0.5183	54.33	93.88	82.14	79.86	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	Creatine	Gluconate	
40	9	0.5177	55.91	93.88	82.56	80.42	BMI	Fasting Insulin	.Galactonate	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	.Linolyl.LPC	Creatine	Gluconate
41	9	0.5183	54.33	93.88	82.14	79.86	BMI	Fasting Insulin	Pyruvate	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	.Linolyl.LPC	Creatine	Gluconate
42	7	0.5390	62.99	93.88	84.21	83.03	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	.Gamma.Glu. Leu	2Hydroxybut yrate	Gluconate		
43	7	0.5382	62.20	93.88	84.04	82.73	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate		
44	8	0.5479	61.42	93.88	83.87	82.44	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Glul.Val	.Linoleate	2Hydroxybut yrate	
45	8	0.5513	59.84	93.88	83.52	81.85	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	
46	9	0.5595	61.42	93.88	83.87	82.44	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Betaine	.Gamma.Glu. Leu	.Linoleate	2Hydroxybut yrate

47	9	0.5575	59.06	93.88	83.33	81.56	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	Hepadecenate
48	9	0.4936	56.69	93.47	81.82	80.63	BMI	Fasting Insulin	2Hydroxybut yrate	Linolenate	Oleate	Hepadecenate	Linoleate	Serine	Threonine
49	9	0.5198	55.91	93.47	81.61	80.35	BMI	Fasting Insulin	2Hydroxybut yrate	Linolyl.LPC	Betaine	Glul.Val	Pyruvate	Gluconate	Galactonate
50	9	0.5211	58.27	93.47	82.22	81.21	Fasting Insulin	Glutamate	BMI	2Hydroxybut yrate	Glycine	Oleate	Adiponectin	Creatine	Gluconate
51	9	0.5167	58.27	93.47	82.22	81.21	Fasting Insulin	Oleoyl.LPC	Pyruvate	BMI	Galactonate	2Hydroxybut yrate	Oleate	Adiponectin	Gluconate
52	7	0.5148	57.48	93.47	82.02	80.92	BMI	Fasting Insulin	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	Creatine	Gluconate		
53	7	0.5128	55.91	93.47	81.61	80.35	BMI	Fasting Insulin	.Glul.Val	.Betaine	2Hydroxybut yrate	.Linolyl.LPC	Gluconate		
54	7	0.5139	55.12	93.47	81.40	80.07	BMI	Fasting Insulin	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	Creatine	Gluconate		
55	8	0.5188	57.48	93.47	82.02	80.92	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	Creatine	Gluconate	
56	8	0.5194	55.91	93.47	81.61	80.35	BMI	Fasting Insulin	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	.Linolyl.LPC	Creatine	Gluconate	
57	8	0.5178	54.33	93.47	81.18	79.79	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	Gluconate	Threonine	
58	9	0.5188	57.48	93.47	82.02	80.92	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	.Oleate	Gluconate	Hepadecenate
59	9	0.5194	55.91	93.47	81.61	80.35	BMI	Fasting Insulin	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Glycine	.Linolyl.LPC	Creatine	Gluconate
60	9	0.5178	54.33	93.47	81.18	79.79	BMI	Fasting Insulin	Pyruvate	.Betaine	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	Creatine	Gluconate
61	7	0.5396	62.99	93.47	83.33	82.97	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Gamma.Glu. Leu	.Linoleate	2Hydroxybut yrate		
62	8	0.5547	62.99	93.47	83.33	82.97	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	.Linoleate	2Hydroxybut yrate	
63	8	0.5493	62.20	93.47	83.16	82.67	BMI	Fasting Insulin	Fasting	Fasting FFA	Adiponectin			Hepadecenate	

							Proinsulin				.Gamma.Glu.Leu	2Hydroxybutyrate					
64	8	0.5525	60.63	93.47	82.80	82.08	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	2Hydroxybutyrate	Gluconate			
65	9	0.5615	62.20	93.47	83.16	82.67	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	.Linoleate	2Hydroxybutyrate	Gluconate		
66	9	0.5589	61.42	93.47	82.98	82.37	BMI	Fasting Insulin	Fasting Proinsulin	Fasting FFA	Adiponectin	.Gamma.Glu.Leu	.Linoleate	2Hydroxybutyrate	Linolyl.LPC		
67	7	0.5127	55.12	93.06	80.46	80.00	BMI	Fasting Insulin	.Gamma.Glu.Leu	2Hydroxybutyrate	.Glycine	.Linolyl.LPC	Creatine				
68	8	0.5179	55.91	93.06	80.68	80.28	BMI	Fasting Insulin	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate	.Glycine	.Linolyl.LPC	Gluconate			
69	9	0.5179	55.91	93.06	80.68	80.28	BMI	Fasting Insulin	.Gamma.Glu.Leu	2Hydroxybutyrate	.Glycine	.Linolenate	.Linolyl.LPC	Creatine	Gluconate		
70	7	0.5388	57.48	93.06	81.11	80.85	BMI	Fasting Insulin	Fasting FFA	Adiponectin	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate				
71	9	0.5081	59.06	93.06	81.52	81.43	Fasting Insulin	Fasting_C_Peptide	BMI	LDL Cholesterol	Galactonate	2Hydroxybutyrate	Oleate	Adiponectin	Gluconate		
72	7	0.5157	56.69	92.65	80.00	80.50	BMI	Fasting Insulin	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate	.Linolyl.LPC	Gluconate				
73	7	0.5124	56.69	92.65	80.00	80.50	BMI	Fasting Insulin	.Betaine	.Galactonate	.Gamma.Glu.Leu	2Hydroxybutyrate	Gluconate				
74	7	0.5131	55.91	92.65	79.78	80.21	BMI	Fasting Insulin	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate	.Glycine	Gluconate				
75	7	0.5121	55.91	92.65	79.78	80.21	BMI	Fasting Insulin	Pyruvate	.Gamma.Glu.Leu	2Hydroxybutyrate	.Linolyl.LPC	Gluconate				
76	8	0.5187	58.27	92.65	80.43	81.07	BMI	Fasting Insulin	.Betaine	.Galactonate	.Gamma.Glu.Leu	2Hydroxybutyrate	.Linolyl.LPC	Gluconate			
77	8	0.5183	55.12	92.65	79.55	79.93	BMI	Fasting Insulin	Pyruvate	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate	.Linolyl.LPC	Gluconate			
78	8	0.5176	55.12	92.65	79.55	79.93	BMI	Fasting Insulin	.Betaine	.Gamma.Glu.Leu	2Hydroxybutyrate	.Linolenate	.Linolyl.LPC	Gluconate			
79	9	0.5187	58.27	92.65	80.43	81.07	BMI	Fasting Insulin	Pyruvate	.Betaine	.Galactonate		.Linolyl.LPC	Gluconate			

80	9	0.5183	55.12	92.65	79.55	79.93	BMI	Fasting Insulin	.Gamma.Glu. Leu	2Hydroxybut yrate	Glycine	.Linolyl.LPC	Creatine	Gluconate	Threonine
81	9	0.5176	55.12	92.65	79.55	79.93	BMI	Fasting Insulin	.Betaine	.Galactonate	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC	Creatine	Gluconate
82	7	0.5383	57.48	92.65	80.22	80.78	BMI	Fasting Insulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	.Linoleate	2Hydroxybut yrate		
83	9	0.5071	55.91	92.65	79.78	80.21	BMI	Fasting Insulin	2Hydroxybut yrate	Oleoyl.LPC	Gluconate	Oleate	Galactonate	Hepadecenate	Linoleate
84	9	0.5137	58.27	92.65	80.43	81.07	Fasting Insulin	Oleoyl.LPC	BMI	Galactonate	Linoleate	2Hydroxybut yrate	Oleate	Adiponectin	Gluconate
85	9	0.5153	53.54	92.65	79.07	79.37	Fasting Insulin	BMI	Galactonate	Linoleate	2Hydroxybut yrate	Glutamate	Oleate	Adiponectin	Gluconate
86	9	0.4879	56.69	92.24	79.12	80.43	BMI	Fasting Insulin	2Hydroxybut yrate	Lactate	Oleate	Arginine	Hepadecenate	Serine	Threonine
87	9	0.5032	55.91	91.84	78.02	80.07	Fasting Insulin	Fasting_ C_Peptide	Lactate	BMI	LDL_ Cholesterol	Galactonate	Triglycerides	2Hydroxybut yrate	Gluconate
88	9	0.4877	55.91	91.84	78.02	80.07	Fasting Insulin	Lactate	Oleate	Serine	BMI	Arginine	2Hydroxybut yrate	HDL Cholesterol	Threonine
89	7	0.5390	57.48	91.84	78.49	80.65	Fasting Insulin	Fasting Insulin	Fasting FFA	Adiponectin	.Gamma.Glu. Leu	2Hydroxybut yrate	.Linolyl.LPC		
90	9	0.5122	52.87	62.63	86.05	81.47	Fasting Insulin	Fasting Insulin	2Hydroxybut yrate	Creatine	Glutamate	Pyruvate	Gluconate	Glycine	Galactonate

Abbreviations: BMI, Body Mass Index; FFA, Free Fatty Acids; FPG, Fasting Plasma Glucose

2H: Correlated Biomarker Compounds :

[00181] Many biomarker compounds were correlated as shown in Tables 9A and 9B. Table 9A contains the pair-wise correlation analysis of biomarkers identified in Study 1 and Table 9B contains the pair-wise correlation analysis of biomarkers identified in Study 2. Correlated compounds are often mutually exclusive in regression models and thus can be used (i.e. substituted for a correlated compound) in different models that had similar prediction powers as those shown in Table 8 above. This aspect is useful when developing biochemical assays that are targeted to specific biomarkers since certain biomarkers may be more amenable to assay development than other biomarkers.

Table 9A. Correlated Biomarkers in Study 1.

Pairwise Correlation	N	Correlation	P-value	R-square
1,5-anhydroglucitol-1,5 (AG) *Metabolite-11234	112	-0.5242	2.99E-09	0.2748
1,5-anhydroglucitol-1,5 (AG) *Metabolite-11249	112	-0.5041	1.46E-08	0.2541
1,5-anhydroglucitol-1,5 (AG) *Metabolite-11252	112	-0.5114	8.32E-09	0.2615
1,5-anhydroglucitol-1,5 (AG) *Metabolite-12061	112	-0.5521	2.78E-10	0.3049
1,5-anhydroglucitol-1,5 (AG) *Metabolite-12064	112	-0.5046	1.40E-08	0.2546
2-hydroxybutyrate (AHB)*1,5-anhydroglucitol-1,5 (AG)	112	-0.5413	7.19E-10	0.2930
2-hydroxybutyrate (AHB)*2-aminobutyrate	112	0.7651	<0.000	0.5854
2-hydroxybutyrate (AHB)*3-hydroxybutyrate (BHBA)	112	0.6517	7.11E-15	0.4247
2-hydroxybutyrate (AHB)*3-methyl-2-oxobutyrate	112	0.6750	2.22E-16	0.4557
2-hydroxybutyrate (AHB)*3-methyl-2-oxovalerate	112	0.5970	3.71E-12	0.3565
2-hydroxybutyrate (AHB)*4-methyl-2-oxopentanoate	112	0.6599	2.44E-15	0.4355
2-hydroxybutyrate (AHB)*creatine	112	0.5123	7.75E-09	0.2624
2-hydroxybutyrate (AHB)*erythrose	112	0.5156	5.96E-09	0.2659
2-hydroxybutyrate (AHB)*galactonic acid	112	0.7137	<0.000	0.5094
2-hydroxybutyrate (AHB)*gluconate	112	0.5427	6.35E-10	0.2945
2-hydroxybutyrate (AHB)*gondoate-20-1-n-9-	112	0.5765	2.91E-11	0.3323
2-hydroxybutyrate (AHB)*isoleucine	112	0.6025	2.09E-12	0.3630
2-hydroxybutyrate (AHB)*leucine	112	0.6472	1.27E-14	0.4188
2-hydroxybutyrate (AHB)*mannose	112	0.7043	<0.000	0.4960
2-hydroxybutyrate (AHB)*margarate (17:0)	112	0.5270	2.38E-09	0.2777
2-hydroxybutyrate (AHB)*palmitate (16:0)	112	0.5191	4.54E-09	0.2694
2-hydroxybutyrate (AHB)*stearate (18:0)	112	0.5888	8.57E-12	0.3467
2-hydroxybutyrate (AHB)*uridine	112	0.5282	2.15E-09	0.2790
2-hydroxybutyrate (AHB)*valine*	112	0.6705	6.66E-16	0.4496
2-hydroxybutyrate (AHB)*Metabolite-10432	112	0.6826	2.22E-16	0.4660
2-hydroxybutyrate (AHB)*Metabolite-10752	112	0.5221	3.55E-09	0.2726
2-hydroxybutyrate (AHB)*Metabolite-11228	112	0.5810	1.87E-11	0.3376
2-hydroxybutyrate (AHB)*Metabolite-11887	112	0.5314	1.66E-09	0.2824
2-hydroxybutyrate (AHB)*Metabolite-11897	112	0.5037	1.51E-08	0.2537
2-hydroxybutyrate (AHB)*Metabolite-12037	112	0.5768	2.82E-11	0.3327
2-hydroxybutyrate (AHB)*Metabolite-12061	112	0.5053	1.33E-08	0.2553

2-hydroxybutyrate (AHB)*Metabolite-12064	112	0.8857	<0.000	0.7844
2-hydroxybutyrate (AHB)*glutamate	112	0.7745	<0.000	0.5998
2-hydroxybutyrate (AHB)*Metabolite-3100	112	0.5619	1.14E-10	0.3158
2-hydroxybutyrate (AHB)*Metabolite-4055	112	0.6836	<0.000	0.4672
2-hydroxybutyrate (AHB)*Metabolite-6488	112	0.5779	2.54E-11	0.3339
2-hydroxybutyrate (AHB)*Metabolite-6627	112	0.5193	4.44E-09	0.2697
2-hydroxybutyrate (AHB)*Metabolite-9033	112	0.5608	1.27E-10	0.3145
2-hydroxybutyrate (AHB)*Metabolite-9043	112	0.5879	9.46E-12	0.3456
2-hydroxybutyrate (AHB)*Metabolite-9727	112	0.7077	<0.000	0.5009
3-hydroxybutyrate (BHBA)*glutamate	112	0.5506	3.18E-10	0.3032
3-methyl-2-oxobutyrate*palmitate (16:0)	112	0.5683	6.31E-11	0.3230
4-methyl-2-oxopentanoate*palmitate (16:0)	112	0.5424	6.53E-10	0.2942
alpha linolenate (18:3(n-3))*dihomo-alpha-linolenate-20-3-n-3-	112	0.5295	1.94E-09	0.2804
alpha linolenate (18:3(n-3))*gondoate-20-1-n-9-	112	0.7264	<0.000	0.5277
alpha linolenate (18:3(n-3))*linoleate (18:2(n-6))	112	0.7877	<0.000	0.6204
alpha linolenate (18:3(n-3))*n-3-DPA-22-5-n-3-	112	0.5722	4.37E-11	0.3274
alpha linolenate (18:3(n-3))*oleate (18:1(n-9))	112	0.7490	<0.000	0.5610
alpha linolenate (18:3(n-3))*palmitate (16:0)	112	0.7354	<0.000	0.5409
alpha linolenate (18:3(n-3))*palmitoleate (16:1(n-7))	112	0.6224	2.36E-13	0.3874
alpha linolenate (18:3(n-3))*stearate (18:0)	112	0.6939	<0.000	0.4815
alpha linolenate (18:3(n-3))*Metabolite-11365	112	0.5054	1.32E-08	0.2555
alpha linolenate (18:3(n-3))*Metabolite-11379	112	0.7245	0	0.5249
alpha linolenate (18:3(n-3))*Metabolite-11653	112	0.5578	1.66E-10	0.3112
alpha linolenate (18:3(n-3))*Metabolite-11887	112	0.7730	0	0.5975
alpha linolenate (18:3(n-3))*Metabolite-12037	112	0.6665	1.11E-15	0.4443
BMI*gamma-glutamylleucine	112	0.5215	3.74E-09	0.2719
BMI*glutamylvaline	112	0.5425	6.47E-10	0.2943
bradykinin*bradykinin, hydroxyproline form-	112	0.5212	3.83E-09	0.2716
creatine*Metabolite-02546_200	112	-0.5849	1.27E-11	0.3421
dipalmitin*palmitate (16:0)	112	0.5630	1.04E-10	0.3170
erythrose*1,5-anhydroglucitol-1,5 (AG)	112	-0.5099	9.35E-09	0.2600
erythrose*galactonic acid	112	0.6691	6.66E-16	0.4476
erythrose*gluconate	112	0.6461	1.44E-14	0.4174
erythrose*glutamate	112	0.7334	0	0.5378
fructose*galactonic acid	112	0.6151	5.35E-13	0.3784
fructose*gluconate	112	0.7100	0	0.5042
fructose*glutamate	112	0.6623	1.78E-15	0.4387
galactonic acid*1,5-anhydroglucitol-1,5 (AG)	112	-0.6211	2.77E-13	0.3857
galactonic acid*2-aminobutyrate	112	0.5246	2.91E-09	0.2752
galactonic acid*3-methyl-2-oxobutyrate	112	0.5517	2.89E-10	0.3044
galactonic acid*4-methyl-2-oxopentanoate	112	0.5288	2.05E-09	0.2797
galactonic acid*gluconate	112	0.7653	0	0.5857
galactonic acid*gondoate-20-1-n-9-	112	0.5981	3.33E-12	0.3577
galactonic acid*isoleucine	112	0.5461	4.74E-10	0.2982
galactonic acid*mannose	112	0.8354	0	0.6978
galactonic acid*margarate (17:0)	112	0.5473	4.26E-10	0.2995
galactonic acid*palmitate (16:0)	112	0.5279	2.21E-09	0.2787
galactonic acid*stearate (18:0)	112	0.5986	3.16E-12	0.3583

galactonic acid*Metabolite-10360	112	0.5113	8.39E-09	0.2614
galactonic acid*Metabolite-10432	112	0.6635	1.55E-15	0.4403
galactonic acid*Metabolite-10609	112	0.5803	2.01E-11	0.3367
galactonic acid*Metabolite-10750	112	0.6247	1.83E-13	0.3903
galactonic acid*Metabolite-10752	112	0.6833	0	0.4668
galactonic acid*Metabolite-11228	112	0.6299	9.99E-14	0.3968
galactonic acid*Metabolite-11230	112	0.5353	1.19E-09	0.2866
galactonic acid*Metabolite-11234	112	0.7072	0	0.5002
galactonic acid*Metabolite-11235	112	0.5168	5.43E-09	0.2671
galactonic acid*Metabolite-11242	112	0.5837	1.43E-11	0.3407
galactonic acid*Metabolite-11249	112	0.5771	2.72E-11	0.3331
galactonic acid*Metabolite-11252	112	0.6003	2.65E-12	0.3603
galactonic acid*Metabolite-11258	112	0.5045	1.42E-08	0.2545
galactonic acid*Metabolite-11387	112	0.5520	2.82E-10	0.3047
galactonic acid*Metabolite-11432	112	0.6147	5.64E-13	0.3778
galactonic acid*Metabolite-11434	112	0.6344	5.93E-14	0.4025
galactonic acid*Metabolite-11628	112	0.5803	2.01E-11	0.3367
galactonic acid*Metabolite-11887	112	0.5451	5.16E-10	0.2971
galactonic acid*Metabolite-11897	112	0.6229	2.25E-13	0.3880
galactonic acid*Metabolite-12037	112	0.5794	2.18E-11	0.3357
galactonic acid*Metabolite-12061	112	0.7387	0	0.5456
galactonic acid*Metabolite-12064	112	0.6051	1.59E-12	0.3662
galactonic acid*glutamate	112	0.9200	0	0.8464
galactonic acid*Metabolite-3100	112	0.5343	1.30E-09	0.2855
galactonic acid*Metabolite-4055	112	0.6292	1.09E-13	0.3958
galactonic acid*Metabolite-5847	112	0.6075	1.23E-12	0.3690
galactonic acid*Metabolite-6446	112	0.6367	4.53E-14	0.4053
galactonic acid*Metabolite-6488	112	0.5753	3.26E-11	0.3309
galactonic acid*Metabolite-6506	112	0.5094	9.72E-09	0.2595
galactonic acid*Metabolite-9033	112	0.7427	0	0.5517
galactonic acid*Metabolite-9043	112	0.5751	3.32E-11	0.3307
galactonic acid*Metabolite-9727	112	0.5195	4.37E-09	0.2699
gamma-glutamylleucine*glutamylvaline	112	0.9404	0	0.8844
gamma-glutamylleucine*peptide-HWESASXX	112	0.7379	0	0.5445
gamma-glutamylleucine*peptide-HWESASXXR	112	0.5766	2.87E-11	0.3325
gluconate*1,5-anhydroglucitol-1,5 (AG)	112	-0.6774	2.22E-16	0.4588
gluconate*mannose	112	0.7419	0	0.5504
gluconate*Metabolite-10432	112	0.6367	4.51E-14	0.4054
gluconate*Metabolite-10609	112	0.5759	3.08E-11	0.3316
gluconate*Metabolite-10610	112	0.5098	9.39E-09	0.2599
gluconate*Metabolite-10750	112	0.7024	0	0.4934
gluconate*Metabolite-10752	112	0.6996	0	0.4894
gluconate*Metabolite-11228	112	0.6652	1.33E-15	0.4425
gluconate*Metabolite-11230	112	0.5881	9.21E-12	0.3459
gluconate*Metabolite-11231	112	0.5374	1.00E-09	0.2888
gluconate*Metabolite-11234	112	0.7041	0	0.4958
gluconate*Metabolite-11235	112	0.5807	1.92E-11	0.3372
gluconate*Metabolite-11242	112	0.6321	7.75E-14	0.3996
gluconate*Metabolite-11249	112	0.5309	1.72E-09	0.2819

gluconate*Metabolite-11252	112	0.5145	6.51E-09	0.2647
gluconate*Metabolite-11387	112	0.5015	1.78E-08	0.2515
gluconate*Metabolite-11432	112	0.5697	5.56E-11	0.3245
gluconate*Metabolite-11434	112	0.6693	6.66E-16	0.4480
gluconate*Metabolite-11435	112	0.5294	1.96E-09	0.2802
gluconate*Metabolite-11628	112	0.5278	2.24E-09	0.2785
gluconate*Metabolite-11897	112	0.6460	1.47E-14	0.4173
gluconate*Metabolite-12061	112	0.7448	0	0.5548
gluconate*Metabolite-12064	112	0.5160	5.78E-09	0.2663
gluconate*glutamate	112	0.7895	0	0.6234
gluconate*Metabolite-3100	112	0.5649	8.70E-11	0.3191
gluconate*Metabolite-4055	112	0.5887	8.67E-12	0.3466
gluconate*Metabolite-4986	112	0.5742	3.63E-11	0.3297
gluconate*Metabolite-5847	112	0.5735	3.88E-11	0.3289
gluconate*Metabolite-6446	112	0.6913	0	0.4779
gluconate*Metabolite-6488	112	0.5778	2.55E-11	0.3339
gluconate*Metabolite-9033	112	0.7458	0	0.5562
gluconate*Metabolite-9043	112	0.5257	2.64E-09	0.2764
glutamylvaline*peptide-HWESASXX	112	0.7816	0	0.6109
glutamylvaline*peptide-HWESASXXR	112	0.5946	4.75E-12	0.3536
glycerate*threonate	112	0.6260	1.57E-13	0.3919
glycerol*alpha linolenate (18:3(n-3))	112	0.6345	5.86E-14	0.4026
glycerol*linoleate (18:2(n-6))	112	0.7008	0	0.4911
glycerol*oleate (18:1(n-9))	112	0.7338	0	0.5385
glycerol*palmitate (16:0)	112	0.6943	0	0.4821
gondoate-20-1-n-9-*linoleate (18:2(n-6))	112	0.7862	0	0.6181
gondoate-20-1-n-9-*oleate (18:1(n-9))	112	0.8756	0	0.7666
gondoate-20-1-n-9-*palmitate (16:0)	112	0.8128	0	0.6607
lactate*pyruvate	112	0.7722	0	0.5963
lactate*Metabolite-4357	112	0.6960	0	0.4844
lactate*Metabolite-4360	112	0.8576	0	0.7355
lactate*Metabolite-4986	112	0.6386	3.60E-14	0.4078
lactate*Metabolite-5348	112	0.5750	3.35E-11	0.3306
linoleate (18:2(n-6))*n-3-DPA-22-5-n-3-	112	0.5966	3.86E-12	0.3560
linoleate (18:2(n-6))*oleate (18:1(n-9))	112	0.8621	0	0.7433
linoleate (18:2(n-6))*palmitate (16:0)	112	0.8248	0	0.6803
linoleate (18:2(n-6))*palmitoleate (16:1(n-7))	112	0.6826	2.22E-16	0.4659
linoleate (18:2(n-6))*stearate (18:0)	112	0.7065	0	0.4991
linoleate (18:2(n-6))*Metabolite-11379	112	0.7858	0	0.6175
linoleate (18:2(n-6))*Metabolite-11653	112	0.5638	9.63E-11	0.3179
linoleate (18:2(n-6))*Metabolite-11887	112	0.8301	0	0.6891
linoleate (18:2(n-6))*Metabolite-12037	112	0.7066	0	0.4992
mannose*1,5-anhydroglucitol-1,5 (AG)	112	-0.6115	8.00E-13	0.3739
mannose*margarate (17:0)	112	0.5273	2.33E-09	0.2780
mannose*glutamate	112	0.8909	0	0.7937
margarate (17:0)*creatine	112	0.5567	1.85E-10	0.3099
margarate (17:0)*stearate (18:0)	112	0.5258	2.63E-09	0.2765
margarate (17:0)*Metabolite-10750	112	0.5003	1.94E-08	0.2503
margarate (17:0)*Metabolite-12037	112	0.5123	7.75E-09	0.2624

margarate (17:0)*Metabolite-12064	112	0.5374	1.00E-09	0.2888
margarate (17:0)*glutamate	112	0.5499	3.38E-10	0.3024
margarate (17:0)*Metabolite-6446	112	0.5082	1.07E-08	0.2582
margarate (17:0)*Metabolite-9033	112	0.5051	1.35E-08	0.2551
myristate (14:0)*oleate (18:1(n-9))	112	0.5118	8.05E-09	0.2619
myristate (14:0)*palmitate (16:0)	112	0.6230	2.22E-13	0.3881
n-3-DPA-22-5-n-3-*oleate (18:1(n-9))	112	0.6538	5.55E-15	0.4275
n-3-DPA-22-5-n-3-*palmitate (16:0)	112	0.6285	1.18E-13	0.3950
oleate (18:1(n-9))*palmitate (16:0)	112	0.9032	0	0.8158
oleate (18:1(n-9))*palmitoleate (16:1(n-7))	112	0.7218	0	0.5209
oleate (18:1(n-9))*stearate (18:0)	112	0.7798	0	0.6081
oleate (18:1(n-9))*Metabolite-11252	112	0.5052	1.34E-08	0.2552
oleate (18:1(n-9))*Metabolite-11379	112	0.8566	0	0.7337
oleate (18:1(n-9))*Metabolite-11653	112	0.6108	8.61E-13	0.3731
oleate (18:1(n-9))*Metabolite-11887	112	0.8989	0	0.8081
oleate (18:1(n-9))*Metabolite-12037	112	0.8335	0	0.6947
ornithine*EDTA*	112	0.6101	9.31E-13	0.3722
ornithine*Metabolite-10812	112	0.5121	7.86E-09	0.2623
ornithine*Metabolite-3091	112	0.5529	2.59E-10	0.3057
ornithine*Metabolite-3103	112	0.5739	3.73E-11	0.3293
ornithine*Metabolite-3108	112	0.5599	1.37E-10	0.3135
ornithine*Metabolite-4274	112	0.5198	4.29E-09	0.2702
palmitate (16:0)*palmitoleate (16:1(n-7))	112	0.7277	0	0.5296
palmitate (16:0)*stearate (18:0)	112	0.8313	0	0.6911
palmitate (16:0)*Metabolite-11252	112	0.5102	9.13E-09	0.2603
palmitate (16:0)*Metabolite-11379	112	0.8594	0	0.7386
palmitate (16:0)*Metabolite-11653	112	0.6739	4.44E-16	0.4542
palmitate (16:0)*Metabolite-11887	112	0.8789	0	0.7724
palmitate (16:0)*Metabolite-12037	112	0.7972	0	0.6356
palmitate (16:0)*Metabolite-12064	112	0.5431	6.13E-10	0.2950
palmitoleate (16:1(n-7))*Metabolite-11379	112	0.8744	0	0.7646
stearate (18:0)*Metabolite-11249	112	0.5247	2.88E-09	0.2753
stearate (18:0)*Metabolite-11252	112	0.5606	1.30E-10	0.3142
stearate (18:0)*Metabolite-11258	112	0.5185	4.74E-09	0.2689
stearate (18:0)*Metabolite-11379	112	0.6025	2.10E-12	0.3630
stearate (18:0)*Metabolite-11653	112	0.5560	1.97E-10	0.3091
stearate (18:0)*Metabolite-11887	112	0.7628	0	0.5818
stearate (18:0)*Metabolite-12037	112	0.7230	0	0.5228
stearate (18:0)*Metabolite-12064	112	0.5859	1.15E-11	0.3433
Metabolite-10432*1,5-anhydroglucitol-1,5 (AG)	112	-0.5674	6.88E-11	0.3220
Metabolite-10432*glutamate	112	0.7402	0	0.5479
Metabolite-10609*glutamate	112	0.5732	3.99E-11	0.3285
glycine*Metabolite-3003	112	0.9861	0	0.9725
Metabolite-10750*1,5-anhydroglucitol-1,5 (AG)	112	-0.5347	1.26E-09	0.2859
Metabolite-10750*glutamate	112	0.6864	0	0.4711
Metabolite-10752*1,5-anhydroglucitol-1,5 (AG)	112	-0.5387	8.96E-10	0.2902
Metabolite-10752*glutamate	112	0.7503	0	0.5629
Metabolite-10814*glutamate	112	0.5121	7.85E-09	0.2623
serine*Metabolite-3078	112	0.6279	1.26E-13	0.3943

serine*Metabolite-3088	112	0.5175	5.15E-09	0.2678
serine*Metabolite-4364	112	0.5899	7.72E-12	0.3480
serine*Metabolite-4769	112	0.6668	1.11E-15	0.4447
glutamate*1,5-anhydroglucitol-1,5 (AG)	112	-0.6945	0	0.4823
glutamate*2-aminobutyrate	112	0.5296	1.92E-09	0.2805
glutamate*3-methyl-2-oxobutyrate	112	0.5857	1.18E-11	0.3430
glutamate*4-methyl-2-oxopentanoate	112	0.5366	1.07E-09	0.2879
glutamate*gondoate-20-1-n-9-	112	0.5823	1.65E-11	0.3390
glutamate*isoleucine	112	0.5442	5.59E-10	0.2961
glutamate*leucine	112	0.5311	1.70E-09	0.2820
glutamate*palmitate (16:0)	112	0.5134	7.08E-09	0.2636
glutamate*stearate (18:0)	112	0.5742	3.63E-11	0.3297
glutamate*valine*	112	0.5511	3.04E-10	0.3037
glutamate*Metabolite-11228	112	0.6659	1.11E-15	0.4435
glutamate*Metabolite-11230	112	0.5964	3.98E-12	0.3557
glutamate*Metabolite-11231	112	0.5182	4.85E-09	0.2686
glutamate*Metabolite-11234	112	0.7245	0	0.5249
glutamate*Metabolite-11235	112	0.5706	5.09E-11	0.3256
glutamate*Metabolite-11242	112	0.6197	3.21E-13	0.3841
glutamate*Metabolite-11249	112	0.6040	1.80E-12	0.3648
glutamate*Metabolite-11252	112	0.6137	6.24E-13	0.3767
glutamate*Metabolite-11258	112	0.5232	3.24E-09	0.2738
glutamate*Metabolite-11387	112	0.5860	1.14E-11	0.3434
glutamate*Metabolite-11432	112	0.6245	1.87E-13	0.3900
glutamate*Metabolite-11434	112	0.6707	6.66E-16	0.4498
glutamate*Metabolite-11435	112	0.5301	1.84E-09	0.2810
glutamate*Metabolite-11628	112	0.5606	1.30E-10	0.3142
glutamate*Metabolite-11887	112	0.5450	5.21E-10	0.2970
glutamate*Metabolite-11897	112	0.6695	6.66E-16	0.4482
glutamate*Metabolite-12037	112	0.5761	3.01E-11	0.3319
glutamate*Metabolite-12061	112	0.7919	0	0.6271
glutamate*Metabolite-12064	112	0.6742	4.44E-16	0.4545
glutamate*Metabolite-3078	112	-0.5143	6.62E-09	0.2645
glutamate*Metabolite-3100	112	0.6438	1.91E-14	0.4145
glutamate*Metabolite-4055	112	0.7284	0	0.5305
glutamate*Metabolite-4986	112	0.5229	3.34E-09	0.2734
glutamate*Metabolite-5847	112	0.6457	1.51E-14	0.4170
glutamate*Metabolite-6446	112	0.7149	0	0.5111
glutamate*Metabolite-6488	112	0.6599	2.44E-15	0.4355
glutamate*Metabolite-6627	112	0.5376	9.88E-10	0.2890
glutamate*Metabolite-9033	112	0.8164	0	0.6665
glutamate*Metabolite-9043	112	0.6741	4.44E-16	0.4544
glutamate*Metabolite-9299	112	0.5571	1.78E-10	0.3103
glutamate*Metabolite-9727	112	0.5597	1.41E-10	0.3132
Metabolite-3078*1,5-anhydroglucitol-1,5 (AG)	112	0.5166	5.54E-09	0.2668
Metabolite-3078*gamma-glutamylglutamine	112	0.5975	3.54E-12	0.3570
Metabolite-4055*1,5-anhydroglucitol-1,5 (AG)	112	-0.5505	3.20E-10	0.3031
pyruvate*Metabolite-4357	112	0.5054	1.32E-08	0.2554
pyruvate*Metabolite-4360	112	0.7299	0	0.5328

Metabolite-4769*gamma-glutamylglutamine	112	0.6458	1.49E-14	0.4171
Metabolite-5847*1,5-anhydroglucitol-1,5 (AG)	112	-0.5083	1.05E-08	0.2584
Metabolite-6446*1,5-anhydroglucitol-1,5 (AG)	112	-0.5887	8.71E-12	0.3465
Metabolite-9033*1,5-anhydroglucitol-1,5 (AG)	112	-0.5674	6.87E-11	0.3220
Metabolite-9033*Metabolite-11228	112	0.6119	7.64E-13	0.3744
Metabolite-9033*Metabolite-11230	112	0.6313	8.50E-14	0.3986
Metabolite-9033*Metabolite-11231	112	0.5342	1.31E-09	0.2854
Metabolite-9033*Metabolite-11234	112	0.6833	0	0.4669
Metabolite-9033*Metabolite-11235	112	0.5267	2.44E-09	0.2774
Metabolite-9033*Metabolite-11242	112	0.5712	4.84E-11	0.3262
Metabolite-9033*Metabolite-11249	112	0.5127	7.51E-09	0.2629
Metabolite-9033*Metabolite-11252	112	0.5285	2.10E-09	0.2794
Metabolite-9033*Metabolite-11387	112	0.5259	2.60E-09	0.2766
Metabolite-9033*Metabolite-11432	112	0.6192	3.42E-13	0.3834
Metabolite-9033*Metabolite-11434	112	0.6631	1.55E-15	0.4396
Metabolite-9033*Metabolite-11435	112	0.5165	5.56E-09	0.2668
Metabolite-9033*Metabolite-11628	112	0.5228	3.36E-09	0.2733
Metabolite-9033*Metabolite-11897	112	0.6761	2.22E-16	0.4572
Metabolite-9033*Metabolite-12061	112	0.7544	0	0.5691
Metabolite-9033*Metabolite-12064	112	0.5456	4.93E-10	0.2977
Metabolite-9033*Metabolite-9043	112	0.5697	5.53E-11	0.3246
Metabolite-9033*Metabolite-9045	112	0.5596	1.42E-10	0.3131
Metabolite-9033*Metabolite-9299	112	0.6908	0	0.4772

Table 9B. Correlated Biomarkers in Study 2.

Compounds	N	Correlation	R-square	p-values
HDL_Cholesterol*Adiponectin	397	0.511148	0.261272	<0.001
Fat_Mass*BMI	402	0.843078	0.710781	<0.001
Weight*BMI	402	0.804681	0.647512	<0.001
Waist*BMI	398	0.800452	0.640724	<0.001
Hip*BMI	398	0.705318	0.497473	<0.001
Fat_Mass_.pcnt*BMI	402	0.602829	0.363403	<0.001
BMI*HOMA	388	0.590842	0.349094	<0.001
BMI*Fasting_Insulin	388	0.589749	0.347804	<0.001
BMI*QUICKI	388	-0.580267	0.336710	<0.001
RD*BMI	402	-0.551166	0.303784	<0.001
BMI*Fasting_C_Peptide	401	0.542661	0.294480	<0.001
Fasting_C_Peptide*HOMA	388	0.829625	0.688277	<0.001
Fasting_Insulin*Fasting_C_Peptide	388	0.828392	0.686233	<0.001
Fasting_C_Peptide*QUICKI	388	-0.768811	0.591070	<0.001
Fasting_Proinsulin*Fasting_C_Peptide	398	0.570761	0.325768	<0.001
Fat_Mass*Fasting_C_Peptide	401	0.519632	0.270017	<0.001
RD*Fasting_C_Peptide	401	-0.506727	0.256773	<0.001
Waist*Fasting_C_Peptide	397	0.501492	0.251495	<0.001
Fasting_Insulin*HOMA	388	0.979376	0.959178	<0.001
Fasting_Insulin*QUICKI	388	-0.880137	0.774641	<0.001
Fasting_Insulin*Fasting_Proinsulin	386	0.509757	0.259853	<0.001

Fat_Mass*Fasting_Insulin	388	0.576818	0.332719	<0.001
Waist*Fasting_Insulin	384	0.502325	0.252330	<0.001
Fasting_Proinsulin*HOMA	386	0.525130	0.275761	<0.001
Fasting_FFA*palmitate (16:0)	393	0.552703	0.305480	<0.001
Fasting_FFA*oleate (18:1(n-9))	393	0.519978	0.270377	<0.001
Fasting_FFA*linoleate (18:2(n-6))	393	0.504094	0.254111	<0.001
Fasting_FFA*Heptadecenate	393	0.503364	0.253375	<0.001
2-aminobutyrate*2-hydroxybutyrate (AHB)	270	0.526705	0.277419	<0.001
alpha linolenate (18:3(n-3))*Isobar-cis-9-cis-11-trans-11-eicosenoate	270	0.634441	0.402516	<0.001
alpha linolenate (18:3(n-3))*linoleate (18:1(n-9)) (18:2(n-6))	270	0.561647	0.315447	<0.001
alpha linolenate (18:3(n-3))*myristate (14:0)	270	0.656699	0.431254	<0.001
alpha linolenate (18:3(n-3))*myristoleate (18:1(n-9))*14-1-n-5-	270	0.580375	0.336836	<0.001
alpha linolenate (18:3(n-3))*n-3-DPA-22-5-n-3-	270	0.730453	0.533562	<0.001
alpha linolenate (18:3(n-3))*oleate (18:1(n-9))	270	0.576371	0.332204	<0.001
alpha linolenate (18:3(n-3))*palmitate (16:0)	270	0.656120	0.430494	<0.001
alpha linolenate (18:3(n-3))*palmitoleate (16:1(n-7))	270	0.631278	0.398512	<0.001
alpha linolenate (18:3(n-3))*stearate (18:0)	270	0.592125	0.350612	<0.001
alpha linolenate (18:3(n-3))*Metabolite-11261	270	0.545276	0.297326	<0.001
alpha linolenate (18:3(n-3))*Heptadecenate	270	0.645969	0.417276	<0.001
alpha linolenate (18:3(n-3))*Metabolite-11521	270	0.536472	0.287803	<0.001
5-oxoproline*gamma-glutamylleucine	270	0.634304	0.402341	<0.001
aspartate*gamma-glutamylleucine	270	0.673200	0.453199	<0.001
erythronate-*gamma-glutamylleucine	270	0.645586	0.416781	<0.001
gamma-glutamylleucine*gamma-glutamylmethionine-	270	0.624245	0.389682	<0.001
gamma-glutamylleucine*gammaglutamylphenylalanine	270	0.797356	0.635776	<0.001
gamma-glutamylleucine*gamma-glutamylthreonine-	270	0.590454	0.348635	<0.001
gamma-glutamylleucine*gamma-glutamyltyrosine	270	0.709135	0.502873	<0.001
gamma-glutamylleucine*glutamine	270	-0.589607	0.347636	<0.001
gamma-glutamylleucine*glycerate	270	0.515968	0.266223	<0.001
gamma-glutamylleucine*Metabolite-10814	270	0.561643	0.315442	<0.001
gamma-glutamylleucine*Metabolite-11505	270	0.580041	0.336448	<0.001
gamma-glutamylleucine*Metabolite-11560	270	0.544984	0.297008	<0.001
gamma-glutamylleucine*Metabolite-12055	270	0.818261	0.669551	<0.001
gamma-glutamylleucine*Metabolite-3078	270	-0.544729	0.296730	<0.001
gamma-glutamylleucine*Metabolite-3114	270	0.610266	0.372424	<0.001
gamma-glutamylleucine*Glutamate	402	0.813405	0.661627	<0.001
gamma-glutamylleucine*glutamylvaline	270	0.980569	0.961516	<0.001
glucose*mannose	270	0.569026	0.323791	<0.001
glucose*galactonic acid	270	0.612109	0.374677	<0.001
5-oxoproline*gluconate	270	0.519968	0.270367	<0.001
5-oxoproline*Glutamate	270	0.598936	0.358724	<0.001
aspartate*Glutamate	270	0.647610	0.419399	<0.001

erythronate-*Glutamate	270	0.577551	0.333565	<0.001
gamma-glutamylleucine*Glutamate	270	0.702612	0.493664	<0.001
gammaglutamylphenylalanine*Glutamate	270	0.683581	0.467283	<0.001
gamma-glutamylthreonine-*Glutamate	270	0.547683	0.299956	<0.001
gamma-glutamyltyrosine*Glutamate	270	0.656418	0.430885	<0.001
glutamine*Glutamate	270	-0.693796	0.481352	<0.001
glycerate*Glutamate	270	0.514091	0.264289	<0.001
Metabolite-10814*Glutamate	270	0.683648	0.467375	<0.001
Metabolite-11505*Glutamate	270	0.565409	0.319687	<0.001
Metabolite-11560*Glutamate	270	0.596566	0.355891	<0.001
Metabolite-12055*Glutamate	270	0.710157	0.504322	<0.001
Metabolite-3078*Glutamate	270	-0.573092	0.328434	<0.001
Metabolite-3114*Glutamate	270	0.717209	0.514389	<0.001
Glutamate*glutamylvaline	402	0.815543	0.665110	<0.001
5-oxoproline*glutamylvaline	270	0.567314	0.321845	<0.001
aspartate*glutamylvaline	270	0.650190	0.422746	<0.001
erythronate-*glutamylvaline	270	0.648632	0.420723	<0.001
gamma-glutamylmethionine-*glutamylvaline	270	0.682431	0.465712	<0.001
gammaglutamylphenylalanine*glutamylvaline	270	0.748588	0.560384	<0.001
gamma-glutamylthreonine-*glutamylvaline	270	0.613004	0.375774	<0.001
gamma-glutamyltyrosine*glutamylvaline	270	0.669454	0.448169	<0.001
glutamine*glutamylvaline	270	-0.586263	0.343704	<0.001
glycerate*glutamylvaline	270	0.500861	0.250862	<0.001
Metabolite-10814*glutamylvaline	270	0.544487	0.296466	<0.001
Metabolite-11505*glutamylvaline	270	0.571094	0.326149	<0.001
Metabolite-11560*glutamylvaline	270	0.520971	0.271411	<0.001
Metabolite-12055*glutamylvaline	270	0.818203	0.669456	<0.001
Metabolite-3078*glutamylvaline	270	-0.536866	0.288225	<0.001
Metabolite-3114*glutamylvaline	270	0.588849	0.346744	<0.001
Docosatetraenate*Heptadecenate	402	0.731992	0.535812	<0.001
Fasting_FFA*Heptadecenate	393	0.503364	0.253375	<0.001
Heptadecenate*palmitate (16:0)	402	0.902155	0.813884	<0.001
Heptadecenate*margarate (17:0)	402	0.827249	0.684341	<0.001
Heptadecenate*stearate (18:0)	402	0.719541	0.517740	<0.001
Heptadecenate*alpha linolenate (18:3(n-3))	402	0.605486	0.366614	<0.001
Isobar-cis-9-cis-11-trans-11-eicosenoate*Heptadecenate	270	0.717041	0.514147	<0.001
linoleate (18:2(n-6))*Heptadecenate	270	0.696473	0.485075	<0.001
myristate (14:0)*Heptadecenate	270	0.815585	0.665178	<0.001
myristoleate (18:1(n-9))*14-1-n-5-*Heptadecenate	270	0.764373	0.584266	<0.001
n-3-DPA-22-5-n-3-*Heptadecenate	270	0.600981	0.361178	<0.001
oleate (18:1(n-9))*Heptadecenate	270	0.826866	0.683707	<0.001
palmitoleate (16:1(n-7))*Heptadecenate	270	0.891137	0.794126	<0.001
Heptadecenate*Metabolite-11909	270	0.500849	0.250850	<0.001
Linolyl.LPC*Oleoyl.LPC	270	0.503307	0.253318	<0.001
hypoxanthine*lactate	270	0.521393	0.271850	<0.001
dihomo-alpha-alpha linolenate (18:3(n-3))-20-3-n-3-*linoleate (18:2(n-6))	270	0.513066	0.263237	<0.001

Isobar-cis-9-cis-11-trans-11-eicosenoate*linoleate (18:2(n-6))	270	0.614356	0.377433	<0.001
linoleate (18:2(n-6))*myristate (14:0)	270	0.777196	0.604033	<0.001
linoleate (18:2(n-6))*oleate (18:1(n-9))	270	0.764875	0.585034	<0.001
linoleate (18:2(n-6))*palmitate (16:0)	270	0.591405	0.349760	<0.001
linoleate (18:2(n-6))*palmitoleate (16:1(n-7))	270	0.667721	0.445851	<0.001
linoleate (18:2(n-6))*stearate (18:0)	402	0.688839	0.474500	<0.001
Docosatetraenate*linoleate (18:2(n-6))	402	0.718624	0.516421	<0.001
linoleate (18:2(n-6))*margarate (17:0)	402	0.658122	0.433124	<0.001
Docosatetraenate*oleate (18:1(n-9))	402	0.764928	0.585115	<0.001
margarate (17:0)*oleate (18:1(n-9))	270	0.510486	0.260596	<0.001
3-hydroxybutyrate (BHBA)*oleate (18:1(n-9))	270	0.576371	0.332204	<0.001
alpha linolenate (18:3(n-3))*oleate (18:1(n-9))	270	0.736518	0.542459	<0.001
Isobar-cis-9-cis-11-trans-11-eicosenoate*oleate (18:1(n-9))	270	0.777196	0.604033	<0.001
linoleate (18:1(n-9)) (18:2(n-6))*oleate (18:1(n-9))	270	0.709041	0.502739	<0.001
margarate (17:0)*oleate (18:1(n-9))	270	0.668674	0.447124	<0.001
myristate (14:0)*oleate (18:1(n-9))	270	0.587740	0.345438	<0.001
myristoleate (18:1(n-9))*14-1-n-5-*oleate (18:1(n-9))	270	0.907290	0.823175	<0.001
oleate (18:1(n-9))*palmitate (16:0)	270	0.766301	0.587217	<0.001
oleate (18:1(n-9))*palmitoleate (16:1(n-7))	270	0.765960	0.586695	<0.001
oleate (18:1(n-9))*stearate (18:0)	402	0.748928	0.560893	<0.001
pyruvate*Metabolite-4357	270	0.586698	0.344214	<0.001
asparagine*serine	270	0.638729	0.407974	<0.001
ornithine*serine	270	0.656649	0.431187	<0.001
serine*Metabolite-4274	270	0.578680	0.334870	<0.001
dihomo-alpha-alpha linolenate (18:3(n-3))-20-3-n-3-*palmitate (16:0)	270	0.516782	0.267063	<0.001
Isobar-cis-9-cis-11-trans-11-eicosenoate*palmitate (16:0)	270	0.703264	0.494580	<0.001
margarate (17:0)*palmitate (16:0)	270	0.752390	0.566091	<0.001
myristate (14:0)*palmitate (16:0)	270	0.807589	0.652199	<0.001
myristoleate (18:1(n-9))*14-1-n-5-*palmitate (16:0)	270	0.658236	0.433274	<0.001
n-3-DPA-22-5-n-3-*palmitate (16:0)	270	0.553025	0.305836	<0.001
palmitate (16:0)*palmitoleate (16:1(n-7))	270	0.784704	0.615761	<0.001
palmitate (16:0)*stearate (18:0)	270	0.843751	0.711916	<0.001
palmitate (16:0)*Heptadecenate	270	0.851782	0.725532	<0.001
palmitate (16:0)*Docosatetraenate	270	0.533851	0.284997	<0.001
Metabolite-9033*Metabolite-10750	270	0.550669	0.303236	<0.001
bradykinin, hydroxyproline form-*peptide-HWESASXXR	270	0.587635	0.345314	<0.001

2I: Predicting and Monitoring Insulin Resistance:

[00182] The biomarker panel and algorithm will measure insulin resistance (IR) which is a root cause of type 2 diabetes. The results will be presented as an "IR Score™" which

represents the level of insulin resistance of the subject. IR Scores will range from Normal Glucose Tolerance (NGT) through increasing levels (Low, Medium, High) of Impaired Glucose Tolerance (IGT). The IR Score™ will allow the physician to place the patient on the spectrum of glucose tolerance, from normal to high. For example, an IR Score™ of 25 will put the patient in the Low IGT category while an IR Score™ of 80 will put the patient in the High IGT category.

[00183] By determining the IR Score on an annual or semi-annual basis, physicians can monitor a patient's progression toward diabetes. For example, an IR score of 25 was obtained at a first time point, an IR Score of 34 was obtained at a second time point, an IR Score of 40 was obtained at a third time point, an IR Score of 40 was obtained at a third time point, an IR Score of 55 was obtained at a fourth time point, and an IR Score of 80 was obtained at a fourth time point indicating an increase in IR and progression of disease from normal to highly impaired glucose tolerance. Using the biomarkers and algorithm of the instant invention for progression monitoring will guide the physician's decision to implement preventative measures such as dietary restrictions, exercise, or early-stage drug treatment. An example of a report demonstrating the use of the IR Score to monitor IR status over time is shown in Figure 5.

Table 10: IR Score

IR Score	1 to 100	≤ 25 NGT 26 to 50 Low IGT 51 to 75 Medium IGT 76 to 100 High IGT > 100 Type 2 Diabetes

2J: Biomarkers that correlate with glucose tolerance tests

[00184] Another study will be carried out to test the biomarkers discovered in the instant invention with a new cohort and to discover additional biochemical biomarkers that correlate with insulin sensitivity (IS) and insulin resistance (IR) as measured by the hyperinsulinemic euglycemic (HI) clamp (Table 11). Using the following study design, baseline fasting EDTA-plasma samples collected from NGT, IGT, IFG IGT/IFG and diabetic subjects (total = 250) will be analyzed.

Table 11: Summary of Study Subjects

Condition	Number of Subjects
NGT	50
IGT	50
IFG	50
IGT/IFG	50
T2D	50

Abbreviations

NGT: Normal Glucose Tolerant (OGTT, <140 mg/dL or < 7.8 mmol/L)
IGT: Impaired Glucose Tolerant (OGTT, 140-199 mg/dL or 7.8 – 11.0 mmol/L)
IFG: Impaired Fasting Glucose (Fasting plasma glucose, 100-125 mg/dL or 5.6-6.9 mmol/L)
IGT/IFG: IGT and/or IFG
T2D : Type II Diabetes (OGTT, ≥ 200 mg/dL or ≥ 11.1 mmol/L)

Example 3: Biomarkers for Metabolic Syndrome Related Disorders

3A: Biomarkers of Metabolic Syndrome

[00185] Biomarkers were discovered by (1) analyzing plasma and serum samples drawn from different groups of subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the two groups.

[00186] The samples used for the analysis were obtained from 19 Caucasian males aged 18-39, average age of 25.6, that had been diagnosed with metabolic syndrome and 19 healthy, age-matched, Caucasian males.

[00187] T-tests were used to determine differences in the mean levels of metabolites between the two populations (i.e., Metabolic syndrome vs. Healthy controls).

Biomarkers:

[00188] As listed below in Tables 12 and 13, biomarkers were discovered that were differentially present between samples from subjects with Metabolic Syndrome and Control (healthy) subjects.

[00189] Tables 12 and 13 include, for each listed biomarker, the p-value and q-value determined in the statistical analysis of the data concerning the biomarkers and an indication of the mean level in metabolic syndrome, the mean level in the control, and the

percentage difference in the metabolic syndrome mean level as compared to the healthy mean level in plasma (Table 12) and serum (Table 13). The term "Isobar" as used in the tables indicates the compounds that could not be distinguished from each other on the analytical platform used in the analysis (i.e., the compounds in an isobar elute at nearly the same time and have similar (and sometimes exactly the same) quant ions, and thus cannot be distinguished). Comp_ID refers to the compound identification number used as a primary key for that compound in the in-house chemical database. Library indicates the chemical library that was used to identify the compounds. The number 50 refer to the GC library and the number 61 refers to the LC library.

Table 12. Metabolite biomarkers of Metabolic Syndrome in plasma

COMP_ ID	COMPOUND	LIB_ ID	p-value	q-value	Mean_ Metabolic Syndrome	Mean_ Ctrl	% Change Met. Syn. vs Control
22290	2-propylpentanoic acid	50	0.2189	0.329	14.75	0.89	1557%
10715	Metabolite - 2395	61	0.3183	0.3868	6.94	0.83	736%
10327	Metabolite - 2281	61	0.0788	0.2083	1.97	0.72	174%
10092	Metabolite - 2250	61	0.3313	0.3919	1.84	0.74	149%
569	caffeine	61	0.0806	0.2083	1.92	0.78	146%
22054	Metabolite - 8792	50	<0.0001	4.00E-04	1.71	0.76	125%
12796	Metabolite - 3114	50	0.0559	0.1731	1.89	0.89	112%
10286	Metabolite - 2272	61	0.0922	0.2131	2.09	1.01	107%
12751	Metabolite - 3073	50	0.0055	0.0575	3.09	1.6	93%
10672	Metabolite - 2390	61	0.0045	0.0575	1.85	0.97	91%
18369	gamma-glu-leu	61	0.2496	0.3473	2.82	1.49	89%
14715	Metabolite - 3653	61	0.5859	0.5302	2.39	1.28	87%
11056	Metabolite - 2568	61	0.3525	0.3951	3.38	1.84	84%
57	glutamic acid	50	0.0403	0.1575	2.6	1.46	78%
9130	Metabolite - 2139	61	0.0027	0.0473	1.62	0.94	72%
1638	arginine	61	0.0795	0.2083	1.53	0.9	70%
24233	Metabolite - 9855	61	0.327	0.3896	1.59	0.95	67%
22130	DL-3-phenyllactic acid	61	0.1899	0.3052	1.82	1.1	65%
17492	Metabolite - 4906	61	0.1714	0.2843	1.62	0.98	65%
21630	Metabolite - 8402	50	0.0044	0.0575	1.48	0.9	64%
17557	Metabolite - 4929	61	0.0132	0.0946	1.25	0.77	62%
15253	Metabolite - 3832	61	0.4313	0.4494	2.22	1.38	61%
20842	Metabolite - 7765	61	0.2648	0.3613	2.17	1.35	61%
14837	Metabolite - 3707	61	0.8263	0.605	3.14	1.97	59%
3147	xanthine	61	0.0204	0.1174	1.54	0.98	57%
21127	monopalmitin	50	0.0025	0.0473	1.5	0.96	56%
2734	gamma-L-glutamyl-L-tyrosine	61	0.1081	0.2168	1.93	1.24	56%

6413	Metabolite - 1342-possible-phenylacetylglutamine-	61	0.2081	0.3221	1.82	1.17	56%
2132	citrulline	50	0.4298	0.4494	0.34	0.22	55%
20830	Metabolite - 7762	61	0.0195	0.1168	1.43	0.93	54%
15996	aspartate	50	0.2785	0.3701	2.81	1.83	54%
18118	Metabolite - 5346	50	0.016	0.1008	1.52	0.99	54%
15113	Metabolite - 3783	61	0.0978	0.2144	0.85	0.56	52%
7171	Metabolite - 1643	61	0.3158	0.3868	2.06	1.36	51%
19377	Metabolite - 6272	50	0.0031	0.0497	1.16	0.77	51%
16337	Metabolite - 4167	61	0.0333	0.1436	1.41	0.94	50%
12756	Metabolite - 3077	50	4.00E-04	0.045	1.93	1.3	48%
17390	Metabolite - 4806	50	0.0276	0.1362	1.23	0.83	48%
21418	Isobar-56-includes-DL-pipecolic acid-1-amino-1-cyclopentanecarboxylic acid	61	0.1632	0.2797	1.78	1.21	47%
1125	isoleucine	50	0.0994	0.2148	1.13	0.77	47%
6847	Metabolite - 1496	61	0.0238	0.1264	1.45	0.99	46%
12658	Metabolite - 3026	50	0.0062	0.0575	1.66	1.15	44%
18392	theobromine	61	0.4972	0.4818	1.41	0.98	44%
13775	Metabolite - 3370	61	0.001	0.0473	1.52	1.06	43%
7933	Metabolite - 1911	61	0.5673	0.5208	1.44	1.01	43%
22320	Metabolite - 8889	50	0.0244	0.1264	0.72	0.51	41%
27278	Metabolite - 10510	50	0.0213	0.1195	1.48	1.05	41%
11178	Metabolite - 2608	61	0.0065	0.0575	1.24	0.88	41%
12656	Metabolite - 3025	50	0.0025	0.0473	1.59	1.13	41%
18882	taurodeoxycholic acid	61	0.2208	0.3294	1.87	1.33	41%
27513	indole-3-acetic acid	61	0.0439	0.1617	1.36	0.97	40%
13214	Metabolite - 3183-possible-gamma-L-glutamyl-L-phenylalanine	61	0.2025	0.3182	2.06	1.48	39%
1481	inositol-1-phosphate	50	0.0444	0.1617	1.72	1.24	39%
60	leucine	50	0.0726	0.2034	1.12	0.81	38%
12780	Metabolite - 3098	50	0.0023	0.0473	1.67	1.21	38%
12774	Metabolite - 3094	50	0.0057	0.0575	1.19	0.87	37%
1561	alpha-tocopherol	50	0.0599	0.1774	1.31	0.96	36%
12647	Metabolite - 3019	50	0.0024	0.0473	1.5	1.1	36%
17068	Metabolite - 4627	61	0.8349	0.6052	1.66	1.22	36%
12960	Metabolite - 3134	61	0.0558	0.1731	1.25	0.92	36%
9491	Metabolite - 2185	61	0.2076	0.3221	1.18	0.87	36%
9172	Metabolite - 2000	61	0.0197	0.1168	1.15	0.85	35%
1898	proline	61	0.0309	0.1425	1.36	1.01	35%
1299	tyrosine	61	0.0027	0.0473	1.3	0.97	34%
18829	phenylalanine	61	0.0014	0.0473	1.51	1.13	34%
12767	Metabolite - 3087	50	0.3192	0.3868	1.24	0.93	33%
9905	Metabolite - 2231	61	0.0482	0.1629	1.45	1.09	33%
19372	Metabolite - 6269	50	0.0255	0.1288	1.01	0.76	33%
19397	Metabolite - 6326	50	0.016	0.1008	1.38	1.04	33%
1649	valine	50	0.1994	0.3156	1.1	0.83	33%
12222	Metabolite - 2374	50	0.0068	0.0575	1.37	1.04	32%
15140	L-kynurenine	61	0.0123	0.0912	1.33	1.01	32%

5628	Metabolite - 1086	61	0.8915	0.6155	1.95	1.49	31%
5687	Metabolite - 1110	61	0.6883	0.573	1.54	1.18	31%
20699	meso-erythritol	50	0.0466	0.1629	1.29	0.99	30%
15990	L-alpha-glycerophosphorylcholine	61	0.2682	0.3634	1.94	1.49	30%
27718	creatine	61	0.0922	0.2131	1.47	1.13	30%
12609	Metabolite - 2986	50	0.0331	0.1436	1.82	1.4	30%
18476	glycocholic acid	61	0.1687	0.283	1.91	1.47	30%
18010	Metabolite - 5231	61	0.2164	0.329	1.52	1.17	30%
12876	Metabolite - 3125	61	0.0652	0.1905	1.22	0.94	30%
19364	Metabolite - 6246	50	0.0105	0.0809	1.32	1.02	29%
10245	Metabolite - 2269-	61	0.84	0.6052	1.5	1.16	29%
6266	Metabolite - 1286	61	0.092	0.2131	1.59	1.23	29%
15506	choline	61	0.1324	0.2451	1.71	1.33	29%
12639	Metabolite - 3012	50	0.0024	0.0473	1.59	1.24	28%
16518	Metabolite - 4276	50	0.1013	0.2164	1.14	0.89	28%
17512	Metabolite - 4912	61	0.5453	0.5139	2.99	2.34	28%
29817	Metabolite - 10683	50	0.0151	0.1008	1.57	1.23	28%
24076	Metabolite - 9726	50	0.0364	0.1465	1.34	1.06	26%
584	mannose	50	0.1042	0.2168	1.39	1.1	26%
18524	6-hydroxydopamine	50	0.3352	0.3923	1.06	0.84	26%
1126	alanine	50	0.1098	0.2168	0.97	0.77	26%
10629	Metabolite - 2386	61	0.5698	0.5208	1.2	0.96	25%
1301	lysine	50	0.3451	0.3929	1.01	0.81	25%
27256	Metabolite - 10500	50	0.0367	0.1465	1.07	0.86	24%
9024	Metabolite - 2111	61	0.0925	0.2131	0.98	0.79	24%
10746	Isobar-6-includes-valine-betaine	61	0.1592	0.2751	1.47	1.19	24%
12768	Metabolite - 3088	50	0.1478	0.2642	1.85	1.5	23%
1572	glyceric acid	50	0.2335	0.3381	1.6	1.3	23%
12650	Metabolite - 3022	50	0.0772	0.2083	1.45	1.18	23%
22337	Metabolite - 8893	61	0.1269	0.2414	1.08	0.88	23%
10087	Metabolite - 2249	61	0.3326	0.3919	1.36	1.11	23%
1670	urea	50	0.0445	0.1617	1.31	1.07	22%
527	lactate	50	0.2905	0.3718	1.54	1.26	22%
16496	Metabolite - 4251	50	0.5501	0.5161	0.88	0.72	22%
8336	Metabolite - 2005	61	0.2177	0.329	1.29	1.06	22%
1303	malic acid	50	0.754	0.5875	1.07	0.88	22%
15737	hydroxyacetic acid	50	0.0844	0.2083	1.08	0.89	21%
16819	Metabolite - 4496	50	0.0589	0.177	1.2	0.99	21%
1358	octadecanoic acid	50	0.0073	0.0584	1.15	0.95	21%
17665	p-hydroxybenzaldehyde	61	0.0329	0.1436	1.84	1.52	21%
7081	Metabolite - 1609	61	0.3512	0.3951	1.05	0.87	21%
10737	Isobar-1-includes-mannose-fructose-glucose-galactose-alpha-L-sorbopyranose-Inositol-D-allose-D--altrose-D-psicone-L--gulose-allo-inositol	61	0.0589	0.177	1.3	1.08	20%
13557	Metabolite - 3323	61	0.5667	0.5208	1.26	1.05	20%

15122	glycerol	50	0.0822	0.2083	1.21	1.01	20%
16511	Metabolite - 4274	50	0.4771	0.4733	1.15	0.96	20%
1121	heptadecanoic acid	50	0.0531	0.1724	1.23	1.03	19%
11053	Metabolite - 2567	61	0.5088	0.4907	3.1	2.6	19%
22026	1-methylguanidine	50	0.0457	0.1629	1.19	1	19%
25609	Metabolite - 10439	50	0.3468	0.3929	1.63	1.37	19%
12035	nonanate	50	0.0892	0.2131	1.47	1.24	19%
1110	arachidonic acid	50	0.1038	0.2168	1.11	0.94	18%
54	tryptophan	61	0.0487	0.1629	1.28	1.09	17%
15278	Metabolite - 3843	61	0.4117	0.4378	1.15	0.98	17%
27570	Metabolite - 10569	61	0.0063	0.0575	1.09	0.93	17%
30178	Metabolite - 10705	61	0.7541	0.5875	1.64	1.4	17%
63	cholesterol	50	0.0343	0.1446	1.17	1	17%
10551	Metabolite - 2347	61	0.6957	0.573	1.96	1.68	17%
21188	1-stearoyl-rac-glycerol	50	0.4701	0.4687	1.14	0.98	16%
1365	tetradecanoic acid	50	0.071	0.2016	1.15	0.99	16%
5426	Metabolite - 1004	61	0.3901	0.4235	1.08	0.93	16%
19368	Metabolite - 6267	50	0.2381	0.3381	1.59	1.37	16%
27273	Metabolite - 10506	50	0.1692	0.283	1.38	1.19	16%
7029	Metabolite - 1597	61	0.0558	0.1731	1.77	1.53	16%
10156	Metabolite - 2259	61	0.7281	0.5856	1.04	0.9	16%
10700	Metabolite - 2393	61	0.6334	0.5495	2.23	1.93	16%
13142	Metabolite - 3165	61	0.0829	0.2083	1.34	1.16	16%
25602	Metabolite - 10432	50	0.9349	0.6253	2.17	1.88	15%
1431	p-hydroxyphenyllactic acid	50	0.2345	0.3381	1.23	1.07	15%
27271	Metabolite - 10504	50	0.2474	0.3466	1.08	0.94	15%
6398	Metabolite - 1335	61	0.8947	0.6155	1.89	1.66	14%
1336	n-hexadecanoic acid	50	0.1174	0.2297	1.09	0.96	14%
27672	3-indoxyl-sulfate	61	0.4812	0.4752	1.3	1.15	13%
22895	Metabolite - 9299	50	0.7239	0.5856	1.05	0.93	13%
12129	beta-hydroxyisovaleric acid	50	0.3035	0.3814	1.4	1.24	13%
19282	Metabolite - 6126	61	0.8029	0.6047	1.14	1.01	13%
21069	dioctyl-phthalate	50	0.0953	0.2144	1.07	0.95	13%
17064	Metabolite - 4624	50	0.0925	0.2131	1.25	1.11	13%
21128	1-octadecanol	50	0.0486	0.1629	1.08	0.96	13%
18232	Metabolite - 5403	50	0.1348	0.2473	1.19	1.06	12%
15529	Metabolite - 3951	61	0.1081	0.2168	1.29	1.15	12%
27675	4-nitrophenol	61	0.3162	0.3868	1.27	1.14	11%
9216	Metabolite - 2168	61	0.0835	0.2083	1.2	1.08	11%
10750	Isobar-8-includes-anthranilic acid-salicylamide	61	0.2166	0.329	1.2	1.08	11%
7601	Metabolite - 1819	61	0.5864	0.5302	1.2	1.08	11%
1604	uric acid	61	0.2964	0.3747	1.04	0.94	11%
513	creatinine	61	0.1885	0.3052	1.06	0.96	10%
1361	pentadecanoic acid	50	0.2362	0.3381	1.17	1.06	10%
1642	decanoic acid	50	0.3114	0.3866	1.39	1.26	10%
18147	Metabolite - 5367	50	0.0753	0.2083	1.11	1.01	10%
22803	Isobar-66-includes-glycochenodeoxycholic acid-	61	0.41	0.4378	1.69	1.54	10%

	glycodeoxycholic acid						
20267	Metabolite - 7187	61	0.9304	0.6243	2.16	1.97	10%
5531	Metabolite - 1095	61	0.5242	0.5009	0.69	0.63	10%
19363	Metabolite - 6227	50	0.3417	0.3923	1.3	1.19	9%
1105	Linoleic acid	50	0.3425	0.3923	1.07	0.98	9%
17228	Metabolite - 4727	61	0.7215	0.5856	1.56	1.43	9%
1643	fumaric acid	50	0.9687	0.6344	1.46	1.34	9%
16782	Metabolite - 4470	61	0.9699	0.6344	1.12	1.03	9%
1302	methionine	61	0.2524	0.3489	1.27	1.17	9%
13545	Metabolite - 3322	61	0.9964	0.6461	1.93	1.78	8%
12083	D-ribose	50	0.6963	0.573	1.31	1.21	8%
20950	Metabolite - 7846	50	0.4339	0.4499	1.32	1.22	8%
5765	Metabolite - 1142	61	0.6088	0.5395	1.21	1.12	8%
27719	galactonic acid	50	0.6632	0.5631	1.08	1	8%
27409	oleamide	50	0.5882	0.5302	0.95	0.88	8%
1507	palmitoleic acid	50	0.7754	0.5955	1.25	1.16	8%
24077	Metabolite - 9727	50	0.5913	0.5308	1.28	1.19	8%
20489	D-glucose	50	0.0349	0.1446	1.14	1.06	8%
6422	Metabolite - 1320	61	0.1095	0.2168	1.02	0.95	7%
19787	Metabolite - 6746	61	0.2617	0.3594	1.17	1.09	7%
5632	Metabolite - 1138	61	0.156	0.2741	1.03	0.96	7%
8098	Metabolite - 1867	61	0.6653	0.5631	1.03	0.96	7%
30273	Metabolite - 10736	50	0.6061	0.5394	1.18	1.1	7%
19934	inositol	50	0.6428	0.553	1.35	1.26	7%
15676	3-methyl-2-oxovaleric acid	61	0.5389	0.5102	1.21	1.13	7%
18349	DL-indole-3-lactic acid	61	0.8534	0.6083	1.22	1.14	7%
15765	ethylmalonic acid	61	0.7431	0.5875	0.99	0.93	6%
30282	Metabolite - 10744	50	0.4613	0.4621	1.17	1.1	6%
16138	Metabolite - 4080	50	0.8136	0.6047	2.4	2.27	6%
10544	Metabolite - 2329	61	0.9173	0.6207	1.11	1.05	6%
15500	carnitine	61	0.6276	0.5468	0.94	0.89	6%
12645	Metabolite - 3017	50	0.6906	0.573	1.32	1.25	6%
16665	Metabolite - 4364	50	0.7407	0.5875	1.16	1.1	5%
17648	Metabolite - 5007	61	0.886	0.6154	2.2	2.09	5%
15365	sn-Glycerol-3-phosphate	50	0.8826	0.6154	1.8	1.71	5%
10499	Metabolite - 2073	61	0.5624	0.5208	1.02	0.97	5%
12638	Metabolite - 3011	50	0.9472	0.6287	1.31	1.25	5%
12663	Metabolite - 3030	50	0.7315	0.5856	1.55	1.48	5%
10065	Metabolite - 2244	61	0.6638	0.5631	0.95	0.91	4%
1645	n-dodecanoate	50	0.451	0.4584	1.24	1.19	4%
6305	Metabolite - 1254	61	0.7656	0.5901	0.82	0.79	4%
18665	Metabolite - 5728	61	0.6202	0.5451	1.14	1.1	4%
13065	Metabolite - 3146	61	0.6037	0.5394	1.21	1.17	3%
7127	Metabolite - 1616	61	0.9018	0.6171	0.92	0.89	3%
12673	Metabolite - 3040	50	0.638	0.5512	1.29	1.25	3%
59	histidine	50	0.8614	0.6083	1	0.97	3%
27275	Metabolite - 10507	50	0.8966	0.6155	1.36	1.32	3%
12626	Metabolite - 3003	50	0.7594	0.5875	1.1	1.07	3%
17627	Metabolite - 4986	50	0.8711	0.6123	1.23	1.2	2%

13589	Metabolite - 3327	61	0.919	0.6207	1.51	1.48	2%
12894	Metabolite - 2456	61	0.9618	0.6331	1.05	1.03	2%
1648	serine	50	0.8095	0.6047	1.14	1.12	2%
20248	Metabolite - 7177	61	0.7567	0.5875	1.23	1.21	2%
25607	Metabolite - 10437	50	0.9076	0.6182	1.34	1.32	2%
1564	citric acid	50	0.4876	0.4779	0.04	0.04	0%
12726	Metabolite - 3058	50	0.693	0.573	1.15	1.15	0%
12593	Metabolite - 2973	50	0.7576	0.5875	0.43	0.43	0%
14988	Metabolite - 3756	61	0.9471	0.6287	1.1	1.1	0%
10147	Metabolite - 2036	61	0.9971	0.6461	1.26	1.27	-1%
16829	Metabolite - 4503	50	0.8459	0.6069	1.19	1.2	-1%
27411	Metabolite - 10547	61	0.9605	0.6331	1.04	1.05	-1%
1410	1-Hexadecanol	50	0.8407	0.6052	0.97	0.98	-1%
10655	Metabolite - 2388	61	0.7806	0.5973	1.1	1.12	-2%
17327	Metabolite - 4767	50	0.8114	0.6047	1.09	1.11	-2%
12666	Metabolite - 3033-possible-threonine-deriv-	50	0.6852	0.573	1.23	1.26	-2%
1366	trans-4-hydroxyproline	50	0.9284	0.6243	1.09	1.12	-3%
21047	3-methyl-2-oxobutyric-	61	0.6204	0.5451	0.99	1.02	-3%
10825	Metabolite - 2546	61	0.7995	0.6047	0.98	1.01	-3%
16070	Metabolite - 4019	50	0.7173	0.5856	1.19	1.23	-3%
22132	DL-alpha-hydroxyisocaproic acid	61	0.8586	0.6083	1.17	1.21	-3%
17786	aldosterone	61	0.8368	0.6052	1.1	1.14	-4%
30265	Metabolite - 10732	50	0.6895	0.573	2.46	2.55	-4%
19097	Metabolite - 5969	61	0.9094	0.6182	0.83	0.87	-5%
22145	acetyl-L-carnitine	61	0.7304	0.5856	1.14	1.2	-5%
1494	5-oxoproline	50	0.8287	0.605	1.33	1.4	-5%
22309	Metabolite - 8887	61	0.8136	0.6047	1.89	1.99	-5%
6571	Metabolite - 1397	61	0.7119	0.5834	0.93	0.98	-5%
16509	Metabolite - 4273	50	0.4072	0.4378	1.3	1.37	-5%
19623	Metabolite - 6671	50	0.563	0.5208	0.33	0.35	-6%
6517	Metabolite - 1338	61	0.4487	0.4583	0.98	1.04	-6%
12162	Metabolite - 2339	50	0.427	0.4494	0.62	0.66	-6%
5733	Metabolite - 1127	61	0.4107	0.4378	1.2	1.28	-6%
27272	Metabolite - 10505	50	0.2265	0.3355	1.38	1.48	-7%
58	glycine	50	0.4963	0.4818	0.96	1.03	-7%
12777	Metabolite - 3097	50	0.5284	0.5025	3.1	3.33	-7%
17568	Metabolite - 4931	61	0.7564	0.5875	1.05	1.13	-7%
13038	Metabolite - 3143	61	0.2742	0.3667	1.29	1.39	-7%
20299	Metabolite - 7266	50	0.341	0.3923	0.86	0.93	-8%
12720	Metabolite - 3056	61	0.5179	0.4971	1.1	1.19	-8%
12782	Metabolite - 3100	50	0.7853	0.5986	1.94	2.1	-8%
22609	Metabolite - 9047	50	0.8612	0.6083	1.45	1.57	-8%
2761	thyroxine	61	0.3227	0.3868	1.2	1.3	-8%
1284	threonine	50	0.4887	0.4779	0.99	1.08	-8%
22548	Metabolite - 9026	50	0.8228	0.605	1.01	1.11	-9%
6851	Metabolite - 1497	61	0.7431	0.5875	0.69	0.76	-9%
7644	Metabolite - 1831-	61	0.1522	0.2697	1.05	1.16	-9%

22880	Metabolite - 9286	50	0.3811	0.4159	0.38	0.42	-10%
12533	Metabolite - 2915	50	0.0688	0.1983	0.9	1	-10%
25402	Metabolite - 10360	50	0.4214	0.4458	0.7	0.78	-10%
20676	maleic acid	61	0.3809	0.4159	0.62	0.7	-11%
6362	Metabolite - 1323-possible-p-cresol-sulfate	61	0.8874	0.6154	1.22	1.38	-12%
22133	DL-hexanoyl-carnitine	61	0.289	0.3718	0.53	0.6	-12%
17304	Metabolite - 4759	61	0.2874	0.3718	0.98	1.11	-12%
16468	Metabolite - 4236	61	0.1187	0.23	0.87	0.99	-12%
5618	Metabolite - 1085	61	0.3652	0.4028	0.97	1.11	-13%
30555	Metabolite - 10781	61	0.8625	0.6083	1.28	1.47	-13%
6373	Metabolite - 1304	61	0.2945	0.3747	1.18	1.36	-13%
22175	l-aspartyl-l-phenylalanine	61	0.8236	0.605	1.03	1.19	-13%
16071	Metabolite - 4020	50	0.1303	0.2435	0.93	1.08	-14%
19402	Metabolite - 6346	50	0.0066	0.0575	0.97	1.13	-14%
22600	Metabolite - 9043	50	0.3407	0.3923	1.09	1.27	-14%
17330	Metabolite - 4769	50	0.2308	0.3381	0.92	1.08	-15%
2342	serotonin	61	0.4557	0.4609	0.89	1.05	-15%
3127	hypoxanthine	61	0.2372	0.3381	1.54	1.82	-15%
15128	DL-homocysteine	61	0.0794	0.2083	0.81	0.96	-16%
16512	Metabolite - 4275	50	0.2404	0.339	1.04	1.27	-18%
17494	Metabolite - 4907	61	0.9491	0.6287	1.53	1.87	-18%
19370	Metabolite - 6268	50	0.2874	0.3718	0.91	1.12	-19%
14672	Metabolite - 3615	61	0.984	0.6416	0.88	1.09	-19%
577	fructose	50	0.2716	0.3657	0.96	1.19	-19%
11499	Metabolite - 2753	61	0.459	0.4619	0.69	0.86	-20%
6374	Metabolite - 1327	61	0.3602	0.4016	0.94	1.18	-20%
22053	3-hydroxydecanoic acid	61	0.3226	0.3868	1.04	1.31	-21%
27738	threonic acid	50	0.1963	0.3131	1.02	1.29	-21%
542	3-hydroxybutanoic acid	50	0.3099	0.3866	1.24	1.57	-21%
12757	Metabolite - 3078	50	0.1683	0.283	1	1.27	-21%
12781	Metabolite - 3099	50	0.2872	0.3718	2.88	3.67	-22%
24074	Metabolite - 9706	50	0.2808	0.3708	0.85	1.1	-23%
53	glutamine	50	0.0982	0.2144	0.71	0.92	-23%
14239	Metabolite - 3474	61	0.1845	0.3012	1.02	1.33	-23%
12625	Metabolite - 3002	50	0.0973	0.2144	0.68	0.9	-24%
19110	Metabolite - 5978	50	0.1078	0.2168	0.64	0.85	-25%
17540	Metabolite - 4926	61	0.4366	0.4504	2.34	3.13	-25%
22570	Metabolite - 9033	50	0.1374	0.2477	0.25	0.34	-26%
10961	Metabolite - 2561	61	0.5701	0.5208	1.31	1.81	-28%
10604	Metabolite - 2370	61	0.1251	0.2402	0.68	0.95	-28%
5657	Metabolite - 1092	61	0.6524	0.5589	3.89	5.46	-29%
18705	Metabolite - 5768	61	0.158	0.2751	1.03	1.48	-30%
16044	Metabolite - 4005	50	0.0144	0.0992	0.55	0.81	-32%
17091	Metabolite - 4641	61	0.1742	0.2866	0.76	1.12	-32%
10066	Metabolite - 2029	61	0.4461	0.4579	1.19	1.77	-33%
22159	dehydroisoandrosterone-3-sulfate	61	0.0303	0.1425	0.93	1.4	-34%
22649	Metabolite - 9108	50	0.0532	0.1724	0.66	1	-34%

17306	Metabolite - 4760	61	0.098	0.2144	0.82	1.25	-34%
9165	Metabolite - 2150	61	0.1062	0.2168	0.76	1.16	-34%
6239	Metabolite - 1264	61	0.1367	0.2477	2.92	4.84	-40%
10781	Metabolite - 2469	61	0.0231	0.126	0.89	1.51	-41%
10304	Metabolite - 2276	61	0.8813	0.6154	1.28	2.19	-42%
5280	biliverdin	61	0.0289	0.1395	1.19	2.14	-44%
18871	Metabolite - 5848	61	0.0069	0.0575	1.03	1.88	-45%
18702	Metabolite - 5767	61	0.0017	0.0473	0.76	1.45	-48%
12478	Metabolite - 2898	61	0.1285	0.2421	0.86	1.71	-50%
27710	N-acetylglycine	50	0.0049	0.0575	0.78	1.64	-52%
17495	Metabolite - 4908	61	0.044	0.1617	0.94	1.99	-53%
10177	Metabolite - 2039	61	0.0067	0.0575	0.83	1.86	-55%
12306	Metabolite - 2869	61	0.627	0.5468	1.18	2.72	-57%

Table 13. Metabolite biomarkers of Metabolic Syndrome in serum.

COMP_ ID	COMPOUND	LIB_ID	p-value	q-value	Mean_ Metabolic Syndrome	Mean_ Control	% Change Met. Syn. vs Control
19402	Metabolite - 6346	50	0	9.00E-04	0.88	1.07	-18%
22054	Metabolite - 8792	50	0	9.00E-04	1.29	0.56	130%
12663	Metabolite - 3030	50	4.00E-04	0.0304	0.61	0.83	-27%
27710	N-acetylglycine	50	6.00E-04	0.0335	0.56	1.28	-56%
18829	phenylalanine	61	0.0015	0.0629	0.95	0.71	34%
13257	Metabolite - 3218	61	0.0019	0.0675	0.94	0.57	65%
9172	Metabolite - 2000	61	0.0023	0.0675	1.24	0.86	44%
17390	Metabolite - 4806	50	0.0032	0.0827	1.2	0.69	74%
20830	Metabolite - 7762	61	0.005	0.1061	1.21	0.69	75%
10672	Metabolite - 2390	61	0.0051	0.1061	1.05	0.63	67%
13142	Metabolite - 3165	61	0.0066	0.1177	0.99	0.78	27%
18147	Metabolite - 5367	50	0.0068	0.1177	1.05	0.9	17%
19110	Metabolite - 5978	50	0.0075	0.1206	1.08	1.66	-35%
16337	Metabolite - 4167	61	0.0087	0.1269	1.33	0.8	66%
27570	Metabolite - 10569	61	0.0095	0.1269	1.15	0.94	22%
6422	Metabolite - 1320	61	0.0098	0.1269	1.07	0.97	10%
21630	Metabolite - 8402	50	0.0104	0.1269	1.17	0.76	54%
1299	tyrosine	61	0.0113	0.1304	1.26	0.87	45%
9491	Metabolite - 2185	61	0.0119	0.1304	1.14	0.77	48%
18702	Metabolite - 5767	61	0.013	0.132	0.75	1.31	-43%
13775	Metabolite - 3370	61	0.0138	0.132	0.93	0.68	37%
10177	Metabolite - 2039	61	0.014	0.132	0.82	1.59	-48%
7081	Metabolite - 1609	61	0.0171	0.1442	1.02	0.84	21%
18871	Metabolite - 5848	61	0.0171	0.1442	0.86	1.32	-35%
12658	Metabolite - 3026	50	0.0178	0.1442	0.98	0.79	24%
12647	Metabolite - 3019	50	0.0191	0.1442	0.92	0.79	16%
12656	Metabolite - 3025	50	0.0193	0.1442	0.88	0.75	17%
18118	Metabolite - 5346	50	0.0194	0.1442	1.16	0.86	35%

17786	aldosterone	61	0.0209	0.1498	1.14	0.91	25%
27273	Metabolite - 10506	50	0.0239	0.1655	0.77	0.91	-15%
17665	p-hydroxybenzaldehyde	61	0.0265	0.1775	0.7	0.53	32%
6374	Metabolite - 1327	61	0.0277	0.1781	1.23	1.92	-36%
7029	Metabolite - 1597	61	0.0305	0.1781	0.67	0.5	34%
21188	1-stearoyl-rac-glycerol	50	0.0308	0.1781	1.16	0.76	53%
16044	Metabolite - 4005	50	0.0317	0.1781	1.01	1.55	-35%
5727	Metabolite - 1126	61	0.0341	0.1781	1.19	0.96	24%
10737	Isobar-1-includes-mannose-fructose-glucose-galactose-alpha-L-sorbopyranose-Inositol-D-allose-D--altrose-D-psicone-L--gulose-allo-inositol	61	0.0343	0.1781	0.93	0.75	24%
1303	malic acid	50	0.0353	0.1781	1.05	1.23	-15%
57	glutamic acid	50	0.0361	0.1781	1.86	1	86%
14491	Metabolite - 3530	61	0.0362	0.1781	0.66	1.02	-35%
12478	Metabolite - 2898	61	0.0364	0.1781	0.64	1.64	-61%
6266	Metabolite - 1286	61	0.0369	0.1781	1.01	0.79	28%
1638	arginine	61	0.0374	0.1781	1.49	0.81	84%
63	cholesterol	50	0.0378	0.1781	1.11	0.93	19%
15529	Metabolite - 3951	61	0.0386	0.1781	0.98	0.84	17%
15278	Metabolite - 3843	61	0.0403	0.181	1.17	0.8	46%
27275	Metabolite - 10507	50	0.0417	0.181	0.6	0.78	-23%
22159	dehydroisoandrosterone-3-sulfate	61	0.0422	0.181	0.8	1.11	-28%
19397	Metabolite - 6326	50	0.0427	0.181	1.12	0.9	24%
12609	Metabolite - 2986	50	0.044	0.1812	0.88	0.7	26%
10087	Metabolite - 2249	61	0.0468	0.1812	1.21	0.84	44%
19377	Metabolite - 6272	50	0.0472	0.1812	1.24	0.95	31%
1604	uric acid	61	0.0485	0.1812	1.12	0.96	17%
54	tryptophan	61	0.0501	0.1812	0.98	0.82	20%
15140	L-kynurenine	61	0.0507	0.1812	1.11	0.85	31%
12666	Metabolite - 3033-possible-threonine-deriv-	50	0.051	0.1812	0.82	0.95	-14%
21127	monopalmitin	50	0.0517	0.1812	1.31	0.82	60%
10629	Metabolite - 2386	61	0.0529	0.1812	1.08	0.82	32%
1125	isoleucine	50	0.0533	0.1812	1.57	1.05	50%
12035	nonanate	50	0.0542	0.1812	0.75	0.58	29%
60	leucine	50	0.0543	0.1812	1.36	1	36%
12751	Metabolite - 3073	50	0.0549	0.1812	0.74	0.64	16%
12781	Metabolite - 3099	50	0.0549	0.1812	0.52	0.75	-31%
12774	Metabolite - 3094	50	0.0566	0.1828	1.11	0.89	25%
24076	Metabolite - 9726	50	0.059	0.1828	1.09	0.9	21%
6571	Metabolite - 1397	61	0.0593	0.1828	1.18	0.94	26%
10499	Metabolite - 2073	61	0.0594	0.1828	1.22	0.96	27%

27278	Metabolite - 10510	50	0.0598	0.1828	1.05	0.82	28%
513	creatinine	61	0.0623	0.1875	1.15	0.93	24%
18665	Metabolite - 5728	61	0.0632	0.1878	0.97	0.85	14%
18369	gamma-glu-leu	61	0.0679	0.1978	1.96	1.02	92%
19370	Metabolite - 6268	50	0.0685	0.1978	0.79	0.97	-19%
20699	meso-erythritol	50	0.0697	0.1986	1.15	0.89	29%
9130	Metabolite - 2139	61	0.0709	0.1991	1.31	0.92	42%
18392	theobromine	61	0.0727	0.2015	1.24	0.69	80%
1649	valine	50	0.074	0.2015	1.5	1.08	39%
18882	taurodeoxycholic acid	61	0.0747	0.2015	2.13	1.13	88%
27718	creatine	61	0.0798	0.2098	1.28	0.94	36%
27513	indole-3-acetic acid	61	0.0815	0.2098	1.17	0.84	39%
9216	Metabolite - 2168	61	0.0832	0.2098	1.02	0.84	21%
9905	Metabolite - 2231	61	0.0833	0.2098	1.08	0.86	26%
19414	Metabolite - 6350	50	0.0838	0.2098	1.33	1.08	23%
20092	Metabolite - 7050	61	0.0844	0.2098	0.59	0.84	-30%
6435	Metabolite - 1348	61	0.0848	0.2098	2.07	0.99	109%
1648	serine	50	0.09	0.22	0.91	1.09	-17%
13214	Metabolite - 3183-possible-gamma-L-glutamyl-L-phenylalanine	61	0.0918	0.2214	1.4	0.97	44%
22649	Metabolite - 9108	50	0.0927	0.2214	0.93	1.17	-21%
30689	Metabolite - 10790	61	0.0939	0.2217	0.97	1.07	-9%
22548	Metabolite - 9026	50	0.0989	0.2309	2.37	0.98	142%
1358	octadecanoic acid	50	0.1003	0.2317	1.04	0.89	17%
16512	Metabolite - 4275	50	0.1067	0.2438	0.71	0.93	-24%
21069	dioctyl-phthalate	50	0.1079	0.2438	1.34	0.88	52%
12960	Metabolite - 3134	61	0.1094	0.2446	1.27	1.05	21%
22609	Metabolite - 9047	50	0.1248	0.272	0.89	0.42	112%
1898	proline	61	0.1257	0.272	1.12	0.88	27%
1642	decanoic acid	50	0.1266	0.272	0.85	0.74	15%
12726	Metabolite - 3058	50	0.1269	0.272	0.81	0.97	-16%
11974	Metabolite - 2827	61	0.1311	0.2779	1.03	1.24	-17%
20842	Metabolite - 7765	61	0.1349	0.2833	1.61	0.97	66%
17304	Metabolite - 4759	61	0.1386	0.2872	1.47	0.92	60%
15737	hydroxyacetic acid	50	0.1396	0.2872	2.37	0.81	193%
18010	Metabolite - 5231	61	0.1433	0.2919	1.16	0.83	40%
59	histidine	50	0.1489	0.2956	0.87	1.03	-16%
16665	Metabolite - 4364	50	0.1496	0.2956	0.78	0.93	-16%
12757	Metabolite - 3078	50	0.15	0.2956	0.67	0.89	-25%
1640	ascorbic acid	50	0.1509	0.2956	1.02	1.65	-38%
21418	Isobar-56-includes-DL-pipecolic acid-1-amino-1-cyclopentanecarboxylic acid	61	0.1522	0.2956	1.17	0.74	58%
10327	Metabolite - 2281	61	0.1573	0.3028	1.44	0.85	69%
10700	Metabolite - 2393	61	0.1613	0.3032	1	0.8	25%
10961	Metabolite - 2561	61	0.1674	0.3032	0.91	0.72	26%

2734	gamma-L-glutamyl-L-tyrosine	61	0.1684	0.3032	1.26	0.85	48%
27409	oleamide	50	0.169	0.3032	2.13	1.09	95%
6847	Metabolite - 1496	61	0.169	0.3032	1.27	1	27%
18705	Metabolite - 5768	61	0.1693	0.3032	0.68	0.97	-30%
27742	aconitate	61	0.1696	0.3032	1.92	1.06	81%
19368	Metabolite - 6267	50	0.1697	0.3032	0.71	0.8	-11%
30265	Metabolite - 10732	50	0.1716	0.3032	0.65	0.85	-24%
542	3-hydroxybutanoic acid	50	0.1728	0.3032	0.91	1.24	-27%
21128	1-octadecanol	50	0.174	0.3032	1.93	0.91	112%
1126	alanine	50	0.175	0.3032	1.28	1.11	15%
9165	Metabolite - 2150	61	0.1811	0.3111	0.7	0.95	-26%
8098	Metabolite - 1867	61	0.1854	0.3159	1.15	0.93	24%
20299	Metabolite - 7266	50	0.1953	0.3285	0.92	1.04	-12%
12129	beta-hydroxyisovaleric acid	50	0.196	0.3285	0.75	0.67	12%
12626	Metabolite - 3003	50	0.1999	0.3315	0.94	1.04	-10%
18476	glycocholic acid	61	0.201	0.3315	1.75	1.57	11%
12222	Metabolite - 2374	50	0.2083	0.3378	1.04	0.91	14%
1361	pentadecanoic acid	50	0.2083	0.3378	2.3	0.96	140%
53	glutamine	50	0.2096	0.3378	1.02	1.23	-17%
22026	1-methylguanidine	50	0.2146	0.3408	1.01	0.92	10%
7127	Metabolite - 1616	61	0.2149	0.3408	1.02	1.28	-20%
10746	Isobar-6-includes-valine-betaine	61	0.217	0.3408	0.88	0.49	80%
5426	Metabolite - 1004	61	0.2181	0.3408	1.97	1.02	93%
1302	methionine	61	0.2235	0.3428	0.91	0.83	10%
1121	heptadecanoic acid	50	0.2248	0.3428	1.5	0.94	60%
6373	Metabolite - 1304	61	0.2252	0.3428	0.77	1.32	-42%
25602	Metabolite - 10432	50	0.226	0.3428	0.64	0.8	-20%
22145	acetyl-L-carnitine	61	0.2282	0.343	1.07	1.24	-14%
18232	Metabolite - 5403	50	0.2307	0.343	1.02	0.93	10%
1365	tetradecanoic acid	50	0.231	0.343	1.31	0.86	52%
10750	Isobar-8-includes-anthranilic acid-salicylamide	61	0.2353	0.346	0.93	0.81	15%
24233	Metabolite - 9855	61	0.238	0.346	1.27	0.75	69%
6305	Metabolite - 1254	61	0.2381	0.346	2.42	1.05	130%
30555	Metabolite - 10781	61	0.2409	0.3476	0.45	0.67	-33%
569	caffeine	61	0.2479	0.3553	1.57	0.79	99%
12162	Metabolite - 2339	50	0.2496	0.3554	1.41	1.48	-5%
16829	Metabolite - 4503	50	0.2514	0.3554	0.83	0.96	-14%
15113	Metabolite - 3783	61	0.2569	0.3592	1.97	1.34	47%
13211	Metabolite - 3182	61	0.2592	0.3592	1.63	1.05	55%
8336	Metabolite - 2005	61	0.261	0.3592	1.07	0.89	20%
17330	Metabolite - 4769	50	0.261	0.3592	0.79	0.91	-13%
12645	Metabolite - 3017	50	0.2635	0.3603	0.81	0.91	-11%
21047	3-methyl-2-	61	0.2676	0.3635	1	0.86	16%

	oxobutyric-						
5733	Metabolite - 1127	61	0.2766	0.3705	0.84	0.92	-9%
1670	urea	50	0.2793	0.3705	0.99	0.89	11%
1572	glyceric acid	50	0.2799	0.3705	0.9	0.69	30%
1507	palmitoleic acid	50	0.2804	0.3705	0.86	1.07	-20%
1110	arachidonic acid	50	0.2843	0.3716	1.03	0.91	13%
1561	alpha-tocopherol	50	0.2874	0.3733	1.07	0.8	34%
19364	Metabolite - 6246	50	0.2912	0.3759	0.91	0.79	15%
5765	Metabolite - 1142	61	0.2932	0.3761	0.99	0.86	15%
6405	Metabolite - 1338	61	0.3015	0.3844	2.63	0.96	174%
16071	Metabolite - 4020	50	0.3073	0.3884	0.92	1	-8%
16496	Metabolite - 4251	50	0.3085	0.3884	1.54	1.24	24%
12533	Metabolite - 2915	50	0.3102	0.3884	1.1	1.01	9%
10286	Metabolite - 2272	61	0.316	0.3933	1.45	1	45%
8469	Metabolite - 2036-possible-Heme	61	0.3184	0.3939	0.88	0.55	60%
10065	Metabolite - 2244	61	0.325	0.3991	1.02	0.95	7%
19623	Metabolite - 6671	50	0.3265	0.3991	2.43	2.77	-12%
27411	Metabolite - 10547	61	0.3324	0.4023	0.52	0.68	-24%
12777	Metabolite - 3097	50	0.3329	0.4023	0.53	0.7	-24%
18394	theophylline	61	0.3355	0.4031	0.97	0.71	37%
2761	thyroxine	61	0.3418	0.4082	0.86	0.93	-8%
18254	paraxanthine	61	0.3437	0.4082	0.97	0.58	67%
7933	Metabolite - 1911	61	0.3458	0.4083	1.13	0.77	47%
15996	aspartate	50	0.3567	0.417	0.63	0.49	29%
7601	Metabolite - 1819	61	0.3571	0.417	0.77	0.72	7%
1336	n-hexadecanoic acid	50	0.3594	0.4174	1.07	0.92	16%
10147	Metabolite - 2036	61	0.3626	0.4186	0.9	0.99	-9%
5618	Metabolite - 1085	61	0.3702	0.4251	1.35	1.06	27%
15500	carnitine	61	0.377	0.429	1.07	0.97	10%
6413	Metabolite - 1342-possible-phenylacetylglutamine-	61	0.3778	0.429	1.1	0.94	17%
27738	threonic acid	50	0.3822	0.4317	0.89	0.96	-7%
22132	DL-alpha-hydroxyisocaproic acid	61	0.3947	0.4428	1	0.88	14%
17306	Metabolite - 4760	61	0.3968	0.4428	0.74	1.09	-32%
20267	Metabolite - 7187	61	0.399	0.4428	2.07	1.37	51%
14368	Metabolite - 3489	61	0.4005	0.4428	0.84	0.94	-11%
10825	Metabolite - 2546	61	0.405	0.4454	1.13	1.02	11%
6546	Metabolite - 1391	61	0.4074	0.4456	1.12	1.02	10%
27675	4-nitrophenol	61	0.4103	0.4465	0.96	0.88	9%
17064	Metabolite - 4624	50	0.4189	0.4534	0.88	0.92	-4%
20489	D-glucose	50	0.4214	0.4538	0.93	0.95	-2%
20676	maleic acid	61	0.4265	0.4553	1.18	1.33	-11%
13557	Metabolite - 3323	61	0.4306	0.4553	1.3	1.02	27%
10551	Metabolite - 2347	61	0.4307	0.4553	1.97	1.41	40%

15990	L-alpha-glycerophosphorylcholine	61	0.4316	0.4553	0.71	0.58	22%
6517	Metabolite - 1338	61	0.4377	0.4591	0.86	0.8	8%
19097	Metabolite - 5969	61	0.4396	0.4591	0.98	1.08	-9%
22803	Isobar-66-includes-glycochenodeoxycholic acid-glycodeoxycholic acid	61	0.442	0.4593	1.63	1.38	18%
1410	1-Hexadecanol	50	0.4484	0.4635	2.68	1.01	165%
15765	ethylmalonic acid	61	0.4505	0.4635	0.95	0.81	17%
5280	biliverdin	61	0.4559	0.4663	0.86	1.01	-15%
1284	threonine	50	0.4577	0.4663	1.14	1.24	-8%
30273	Metabolite - 10736	50	0.4611	0.4675	0.89	1	-11%
16518	Metabolite - 4276	50	0.4687	0.4717	0.86	0.72	19%
12756	Metabolite - 3077	50	0.4698	0.4717	0.73	0.68	7%
12639	Metabolite - 3012	50	0.4752	0.4748	0.79	0.73	8%
12650	Metabolite - 3022	50	0.4937	0.4894	0.84	0.77	9%
10544	Metabolite - 2329	61	0.4945	0.4894	1.05	1.4	-25%
22337	Metabolite - 8893	61	0.5005	0.493	1.07	1.14	-6%
18524	6-hydroxydopamine	50	0.5075	0.4953	1.13	1.2	-6%
13065	Metabolite - 3146	61	0.5076	0.4953	1.01	0.97	4%
15506	choline	61	0.5148	0.5	1.02	0.75	36%
14988	Metabolite - 3756	61	0.5198	0.5007	0.68	0.74	-8%
12894	Metabolite - 2456	61	0.5228	0.5007	0.9	0.94	-4%
22880	Metabolite - 9286	50	0.5255	0.5007	1.33	1.25	6%
7171	Metabolite - 1643	61	0.5279	0.5007	2.54	2.05	24%
14715	Metabolite - 3653	61	0.5332	0.5007	1.92	1.07	79%
15000	Metabolite - 3758	61	0.5332	0.5007	0.96	0.91	5%
29817	Metabolite - 10683	50	0.5335	0.5007	0.84	0.79	6%
19372	Metabolite - 6269	50	0.535	0.5007	1.09	1.13	-4%
18091	Metabolite - 5306	61	0.5372	0.5007	1.06	0.96	10%
15676	3-methyl-2-oxovaleric acid	61	0.554	0.5065	0.93	0.89	4%
16468	Metabolite - 4236	61	0.5544	0.5065	1.1	0.98	12%
22053	3-hydroxydecanoic acid	61	0.5575	0.5065	0.92	1.07	-14%
10781	Metabolite - 2469	61	0.5584	0.5065	0.8	1	-20%
17568	Metabolite - 4931	61	0.5585	0.5065	0.89	1.05	-15%
12625	Metabolite - 3002	50	0.5605	0.5065	1.74	1.78	-2%
1643	fumaric acid	50	0.5626	0.5065	0.88	0.96	-8%
27272	Metabolite - 10505	50	0.5629	0.5065	0.72	0.74	-3%
19363	Metabolite - 6227	50	0.5725	0.5111	0.83	0.79	5%
10655	Metabolite - 2388	61	0.5733	0.5111	0.93	1	-7%
58	glycine	50	0.5779	0.5111	0.9	0.98	-8%
15128	DL-homocysteine	61	0.5816	0.5111	1.27	1.36	-7%
17068	Metabolite - 4627	61	0.5821	0.5111	1.35	0.76	78%
1494	5-oxoproline	50	0.5828	0.5111	0.99	0.86	15%
6531	Metabolite - 1385	61	0.5892	0.5145	1.09	1.14	-4%

19282	Metabolite - 6126	61	0.605	0.5238	1.38	1.03	34%
22130	DL-3-phenyllactic acid	61	0.6056	0.5238	2.23	1.36	64%
1301	lysine	50	0.6074	0.5238	1.53	1.32	16%
6851	Metabolite - 1497	61	0.616	0.5277	1.55	1.46	6%
6398	Metabolite - 1335	61	0.6169	0.5277	1.34	1.56	-14%
27256	Metabolite - 10500	50	0.6227	0.5304	1.1	1.05	5%
21841	Metabolite - 8577	61	0.6366	0.54	1.08	0.99	9%
5687	Metabolite - 1110	61	0.6394	0.5402	0.96	0.94	2%
12306	Metabolite - 2869	61	0.6482	0.5455	2.58	1.89	37%
12767	Metabolite - 3087	50	0.6549	0.5488	1.44	1.18	22%
12638	Metabolite - 3011	50	0.6612	0.5519	0.91	0.99	-8%
5489	Metabolite - 1057	61	0.6673	0.5547	1.03	1.05	-2%
19462	Metabolite - 6446	50	0.6727	0.5556	1.08	1.04	4%
10604	Metabolite - 2370	61	0.676	0.5556	0.86	0.88	-2%
15121	Metabolite - 3786	61	0.6764	0.5556	0.86	0.89	-3%
10092	Metabolite - 2250	61	0.6803	0.5561	1.63	0.76	114%
22320	Metabolite - 8889	50	0.6822	0.5561	1.69	1.77	-5%
1645	n-dodecanoate	50	0.6858	0.5567	0.82	0.79	4%
527	lactate	50	0.6911	0.5589	0.99	0.91	9%
3127	hypoxanthine	61	0.7003	0.5629	0.54	0.51	6%
24077	Metabolite - 9727	50	0.7015	0.5629	0.92	0.97	-5%
1366	trans-4-hydroxyproline	50	0.7106	0.568	1.14	1.08	6%
11499	Metabolite - 2753	61	0.7138	0.5684	1.31	0.8	64%
18349	DL-indole-3-lactic acid	61	0.7229	0.5722	0.91	0.86	6%
7644	Metabolite - 1831-	61	0.7346	0.5751	0.88	0.89	-1%
5531	Metabolite - 1095	61	0.7348	0.5751	1.34	1.34	0%
12768	Metabolite - 3088	50	0.736	0.5751	0.65	0.68	-4%
1431	p-hydroxyphenyllactic acid	50	0.7405	0.5764	1.19	0.91	31%
12673	Metabolite - 3040	50	0.7654	0.5878	0.75	0.82	-9%
2132	citrulline	50	0.7659	0.5878	3.41	3.29	4%
22309	Metabolite - 8887	61	0.7694	0.5878	0.58	0.54	7%
30282	Metabolite - 10744	50	0.7699	0.5878	0.89	0.87	2%
14672	Metabolite - 3615	61	0.7721	0.5878	0.82	0.89	-8%
25607	Metabolite - 10437	50	0.7765	0.589	0.76	0.87	-13%
6820	Metabolite - 1554	61	0.7865	0.5944	1.06	1	6%
14239	Metabolite - 3474	61	0.7901	0.5949	1	0.9	11%
20248	Metabolite - 7177	61	0.7984	0.5973	1.04	1.02	2%
27326	Metabolite - 10527	50	0.799	0.5973	1.16	1.12	4%
25402	Metabolite - 10360	50	0.8093	0.6029	1.29	1.3	-1%
19934	inositol	50	0.8135	0.6038	0.68	0.68	0%
12593	Metabolite - 2973	50	0.8339	0.6152	1.66	1.66	0%
23462	Metabolite - 9693	61	0.8348	0.6152	1.15	1.15	0%
16819	Metabolite - 4496	50	0.8463	0.6215	0.88	0.85	4%
16138	Metabolite - 4080	50	0.8667	0.6297	0.53	0.51	4%
25609	Metabolite - 10439	50	0.8668	0.6297	0.72	0.66	9%

584	mannose	50	0.87	0.6297	0.93	0.84	11%
15122	glycerol	50	0.8737	0.6297	0.93	0.89	4%
22570	Metabolite - 9033	50	0.875	0.6297	1.52	1.51	1%
20888	Metabolite - 7806	61	0.8756	0.6297	0.97	0.99	-2%
13038	Metabolite - 3143	61	0.8833	0.6316	1.32	1.14	16%
16509	Metabolite - 4273	50	0.8851	0.6316	0.69	0.7	-1%
16511	Metabolite - 4274	50	0.8873	0.6316	1.1	1.08	2%
13589	Metabolite - 3327	61	0.8935	0.6338	1.19	1.06	12%
5628	Metabolite - 1086	61	0.9002	0.6364	1.73	1.6	8%
10245	Metabolite - 2269-	61	0.9081	0.6398	1.08	0.87	24%
6362	Metabolite - 1323-possible-p-cresol-sulfate	61	0.9178	0.6429	1.08	1.02	6%
5632	Metabolite - 1138	61	0.9201	0.6429	1.03	1.05	-2%
15365	sn-Glycerol-3-phosphate	50	0.9265	0.6429	0.82	0.82	0%
10304	Metabolite - 2276	61	0.9274	0.6429	1.36	1.77	-23%
10156	Metabolite - 2259	61	0.9281	0.6429	1.06	1.03	3%
27719	galactonic acid	50	0.9386	0.6449	1.13	1.1	3%
22895	Metabolite - 9299	50	0.9394	0.6449	1.05	1.05	0%
15253	Metabolite - 3832	61	0.9468	0.6449	1.48	1.41	5%
1105	Linoleic acid	50	0.9483	0.6449	0.95	0.94	1%
12780	Metabolite - 3098	50	0.9494	0.6449	0.87	0.83	5%
10441	Metabolite - 2308	61	0.9506	0.6449	0.92	0.88	5%
27672	3-indoxyl-sulfate	61	0.9527	0.6449	0.98	0.95	3%
22133	DL-hexanoyl-carnitine	61	0.9585	0.646	1.36	1.35	1%
16070	Metabolite - 4019	50	0.9631	0.646	0.85	0.85	0%
12720	Metabolite - 3056	61	0.9635	0.646	0.9	0.92	-2%
24074	Metabolite - 9706	50	0.9711	0.6479	1.12	1.11	1%
13545	Metabolite - 3322	61	0.9736	0.6479	1.49	1.34	11%
1564	citric acid	50	0.9794	0.6479	3.72	3.73	0%
17327	Metabolite - 4767	50	0.9803	0.6479	1.32	1.03	28%
20950	Metabolite - 7846	50	0.982	0.6479	0.93	0.97	-4%
14837	Metabolite - 3707	61	0.99	0.6511	2.12	1.66	28%

[00190] Random forests were generated for plasma and serum biomarkers. The models for the serum biomarkers correctly classified 81.5% of the subjects as either being healthy or having metabolic syndrome; 83% of the healthy subjects were classified correctly and 77% of the subjects having metabolic syndrome were correctly classified. For the models based on the biomarkers from plasma, the 89% of the subjects were correctly classified as either being healthy or having metabolic syndrome; 100% of the healthy subjects were correctly classified and 77% of the metabolic syndrome subjects

were correctly classified. The most important biomarkers are shown in the importance plot in Figure 8 (Serum) and Figure 9 (Plasma).

3B: Biomarkers of atherosclerosis

[00191] Biomarkers were discovered by (1) analyzing plasma, aorta and liver samples drawn from subjects with atherosclerosis and healthy subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the two groups.

[00192] The samples used for the analysis were from wild-type and transgenic mice, C57BL/6 and LDb, respectively. The transgenic LDb mice provide a model for atherosclerosis in human subjects. Previous studies have shown that LDb transgenic mice in a C57BL/6 background have about 5-fold higher plasma cholesterol and triglyceride levels than C57BL/6 wild-type mice and start to develop atherosclerotic lesions at about 3 months of age. Plasma, ascending and descending aorta tissues and liver tissue from each group of mice at 2, 5 or 8 months were subjected to metabolomic analysis. These collection time points represent early (initiation), mid and late stage for atherosclerosis in the transgenic model.

[00193] T-tests were used to determine differences in the mean levels of metabolites between the two populations (i.e., LDb vs. C57BL/6). Classification analysis was carried out using recursive partitioning and random forest analyses to uncover the biomarkers that can best differentiate the 2 groups of mice. Recursive partitioning relates a 'dependent' variable (Y) to a collection of independent ('predictor') variables (X) in order to uncover -- or simply understand -- the elusive relationship, Y=f(X). It was performed with the JMP program (SAS) to generate a decision tree. The statistical significance of the "split" of the data can be placed on a more quantitative footing by computing p-values, which discern the quality of a split relative to a random event. The significance level of each "split" of data into the nodes or branches of the tree was computed as p-values, which discern the quality of the split relative to a random event. It was given as LogWorth, which is the negative log10 of a raw p-value. Statistical analyses were performed with the program "R" available on the worldwide web at the website cran.r-project.org.

[00194] Random forests give an estimate of how well individuals can be classified in a

new data set into each group, in contrast to a t-test, which tests whether the unknown means for two populations are different or not. Random forests create a set of classification trees based on continual sampling of the experimental units and compounds. Then each observation is classified based on the majority votes from all the classification trees. Statistical analyses were performed with the program "R" available on the worldwide web at the website cran.r-project.org.

Biomarkers:

[00195] As listed below in Tables 14, 15 and 16, biomarkers were discovered that were differentially present between samples from LDb (atherosclerotic) subjects and C57BL/6 (healthy) subjects.

[00196] Tables 14, 15 and 16 include, for each listed biomarker, the p-value and q-value determined in the statistical analysis of the data concerning the biomarkers and an indication of the percentage difference in the atherosclerotic mean level as compared to the healthy mean level in plasma (Table 14), aorta (Table 15) and liver (Table 16). The term "Isobar" as used in the tables indicates the compounds that could not be distinguished from each other on the analytical platform used in the analysis (i.e., the compounds in an isobar elute at nearly the same time and have similar (and sometimes exactly the same) quant ions, and thus cannot be distinguished). Comp_ID refers to the compound identification number used as a primary key for that compound in the in-house chemical database. Library indicates the chemical library that was used to identify the compounds. The number 50 refer to the GC library and the number 61 refers to the LC library.

Table 14. Metabolite biomarkers of Atherosclerosis in plasma.

COMP_ID	COMPOUND	LIB_ID	p-value	q-value	% Change in LDb
21415	Metabolite - 8209	50	4.25E-07	2.40E-06	497%
21012	Metabolite - 7889	50	1.12E-22	6.95E-21	494%
21011	Metabolite - 7888	50	8.87E-24	8.25E-22	482%
25649	Metabolite - 10450	50	4.17E-15	7.76E-14	442%

18619	Metabolite - 5669	61	5.95E-06	2.84E-05	423%
27279	Metabolite - 10511	50	1.81E-19	6.74E-18	404%
63	cholesterol	50	1.56E-24	2.90E-22	400%
8469	Metabolite - 2036-possible-Heme	61	1.00E-04	4.00E-04	371%
27278	Metabolite - 10510	50	5.00E-19	1.33E-17	351%
22993	Metabolite - 9448	50	4.73E-15	8.00E-14	350%
25366	Metabolite - 10286	61	1.65E-05	1.00E-04	339%
21631	Metabolite - 8403	50	9.85E-21	4.58E-19	306%
27414	beta-sitosterol	50	1.11E-12	1.29E-11	302%
21013	Metabolite - 7890	50	6.00E-14	7.73E-13	237%
12785	Metabolite - 3103	50	0.0011	0.0026	236%
16831	Metabolite - 4504	50	2.00E-04	5.00E-04	234%
18155	Metabolite - 5386	61	2.55E-05	1.00E-04	223%
8159	Metabolite - 1971	61	0.0026	0.0054	213%
27256	Metabolite - 10500	50	2.49E-19	7.72E-18	209%
21127	monopalmitin	50	6.23E-14	7.73E-13	204%
21184	1-oleoyl-rac-glycerol	50	6.14E-08	4.17E-07	196%
6380	Metabolite - 1330	61	0.0963	0.0914	177%
21188	1-stearoyl-rac-glycerol	50	5.43E-12	5.94E-11	177%
22032	Metabolite - 8766	50	1.79E-14	2.78E-13	163%
1561	alpha-tocopherol	50	4.00E-04	0.001	161%
6266	Metabolite - 1286	61	6.00E-04	0.0015	130%
19323	Docosahexaenoic-Acid	50	2.43E-17	5.65E-16	126%
27890	Metabolite - 10611	50	2.81E-09	2.26E-08	126%
6130	Metabolite - 1208	61	1.04E-05	4.62E-05	124%
6362	p-cresol-sulfate	61	2.77E-05	1.00E-04	123%
9172	Metabolite - 2000	61	1.43E-15	2.96E-14	121%
15991	L-alpha-glycerophosphorylcholine	61	0.0014	0.0032	117%
9905	Metabolite - 2231	61	1.00E-04	2.00E-04	116%
15611	Metabolite - 3971	61	3.71E-06	1.82E-05	115%
24205	Metabolite - 9841	61	1.98E-06	1.02E-05	111%
12604	Metabolite - 2981	50	6.77E-09	5.04E-08	111%
27888	Metabolite - 10609	50	0.0116	0.0181	110%
27728	glycerol-2-phosphate	50	8.04E-06	3.65E-05	107%
12035	nonanate	50	3.95E-05	2.00E-04	104%
17251	Metabolite - 4732	61	0.0089	0.0147	103%
23079	Metabolite - 9647	61	1.18E-05	1.00E-04	101%

1110	arachidonic acid	50	0.0015	0.0032	100%
15753	hippuric acid	61	1.00E-04	2.00E-04	97%
17800	cortodoxone	61	1.00E-04	4.00E-04	92%
27773	Isobar-71[1]	61	0.0301	0.0379	92%
1359	oleic acid	50	0.0669	0.0711	90%
1358	octadecanoic acid	50	4.62E-14	6.61E-13	88%
12774	Metabolite - 3094	50	2.92E-09	2.26E-08	86%
12767	Metabolite - 3087	50	0.007	0.0124	85%
24330	Metabolite - 10126	61	1.00E-09	8.46E-09	84%
21150	sinapic acid	61	1.10E-10	9.75E-10	84%
20136	Metabolite - 7062	61	3.65E-05	1.00E-04	80%
10782	Metabolite - 2486	61	0.0854	0.0837	80%
6171	Metabolite - 1244	61	0.003	0.0059	78%
16138	Metabolite - 4080	50	3.02E-07	1.76E-06	77%
21418	Isobar-56[2]	61	6.28E-08	4.17E-07	77%
1105	linoleic acid	50	3.36E-11	3.29E-10	75%
1336	n-hexadecanoic acid	50	3.99E-11	3.71E-10	73%
12112	Metabolite - 2314	61	1.47E-06	7.82E-06	72%
57	glutamic acid	50	0.0083	0.0141	71%
22586	Metabolite - 9039	61	1.00E-04	3.00E-04	70%
21828	Metabolite - 8574	61	0.0027	0.0055	68%
26444	Metabolite - 10465	61	2.89E-11	2.99E-10	66%
5475	Metabolite - 1033	61	1.01E-07	6.06E-07	63%
10700	Metabolite - 2393	61	0.0101	0.0162	61%
22259	Isobar-59 [3]	61	0.0013	0.003	58%
16992	Metabolite - 4603	61	0.0237	0.031	57%
18691	Metabolite - 5749	61	0.0854	0.0837	57%
19934	inositol	50	0.0067	0.012	55%
12609	Metabolite - 2986	50	0.0013	0.0029	55%
15365	sn-Glycerol-3-phosphate	50	8.00E-04	0.0018	53%
11499	Metabolite - 2753	61	0.0071	0.0125	51%
16074	Metabolite - 2758	50	6.00E-04	0.0016	49%
15670	2-methylhippuric acid	61	6.00E-04	0.0016	49%
8210	Metabolite - 1981	61	0.017	0.0238	47%
1121	heptadecanoic acid	50	8.42E-09	6.03E-08	46%
17488	Metabolite - 4887	61	2.00E-04	6.00E-04	45%
1493	ornithine	50	0.0482	0.0557	42%

6851	Metabolite - 1497	61	0.0676	0.0711	42%
22895	Metabolite - 9299	50	0.0133	0.0195	39%
13038	Metabolite - 3143	61	0.0117	0.0181	39%
5699	Metabolite - 1157	61	0.0036	0.0068	37%
18015	Metabolite - 3113	61	1.00E-04	4.00E-04	36%
24076	Metabolite - 9726	50	3.00E-04	0.001	36%
20031	Metabolite - 7007	61	0.0011	0.0026	36%
20488	D-glucose	50	2.00E-04	7.00E-04	34%
10401	Metabolite - 2058	61	0.0015	0.0032	34%
1507	palmitoleic acid	50	0.0168	0.0236	34%
1670	urea	50	4.00E-04	0.001	34%
15948	S-adenosyl-l-homocysteine	61	0.023	0.0303	33%
5465	Metabolite - 1029	61	8.00E-04	0.002	33%
2849	guanosine-5-monophosphate	61	0.0418	0.0499	33%
1494	5-oxoproline	50	0.0039	0.0073	32%
11923	Metabolite - 2821	61	0.0089	0.0147	32%
18969	Metabolite - 5920	61	0.0033	0.0063	32%
20830	Metabolite - 7762	61	0.056	0.0621	31%
2832	adenosine-5-monophosphate	61	0.0137	0.0199	30%
17083	Metabolite - 4634	50	0.0718	0.0742	28%
15996	aspartate	50	0.1053	0.0995	27%
14639	Metabolite - 3603	61	0.0225	0.0302	26%
25514	Metabolite - 10404	61	0.0747	0.0768	26%
17747	D-sphingosine	50	0.0239	0.0311	26%
27137	Metabolite - 10498	50	0.0511	0.0576	26%
30128	Metabolite - 10687	61	1.00E-04	4.00E-04	25%
16244	Isobar-21 [4]	61	0.0038	0.0072	25%
15053	sorbitol	50	0.0718	0.0742	25%
15122	glycerol	50	0.0019	0.004	25%
14387	Metabolite - 3490	61	0.0754	0.0771	23%
1642	decanoic acid	50	0.0455	0.0536	23%
20675	1,5-anhydro-D-glucitol	50	0.1081	0.1016	22%
11438	phosphate	50	0.0022	0.0045	21%
584	mannose	50	0.0128	0.0191	19%
2129	L-5-Hydroxytryptophan	61	0.0809	0.0806	19%
9491	Metabolite - 2185	61	0.0162	0.023	18%

605	uracil	50	0.0468	0.0544	18%
10655	Metabolite - 2388	61	0.0112	0.0177	18%
17665	p-hydroxybenzaldehyde	61	0.0121	0.0186	18%
10746	Isobar-6 [5]	61	0.0564	0.0621	16%
19372	Metabolite - 6269	50	0.0117	0.0181	16%
9216	Metabolite - 2168	61	0.0464	0.0543	15%
15113	Metabolite - 3783	61	0.0126	0.019	14%
27718	creatine	61	0.0245	0.0312	14%
1113	isocitrate	61	0.0132	0.0195	14%
18232	Metabolite - 5403	50	0.0493	0.0562	14%
10737	Isobar-1 [6]	61	0.0229	0.0303	13%
7175	Metabolite - 1655	61	0.0099	0.016	13%
19402	Metabolite - 6346	50	0.0411	0.0496	10%
15677	3-methyl-L-histidine	61	0.0795	0.0799	-8%
9324	Metabolite - 2173	61	0.0842	0.0834	-9%
54	tryptophan	61	0.0808	0.0806	-11%
17007	Metabolite - 4609	61	0.0957	0.0914	-11%
1574	histamine	61	0.0574	0.0629	-12%
5765	Metabolite - 1142	61	0.0951	0.0913	-14%
27672	3-indoxyl-sulfate	61	0.0514	0.0576	-15%
10667	Metabolite - 2389	61	0.0939	0.0906	-15%
15765	ethylmalonic acid	61	0.0241	0.0311	-17%
16705	Metabolite - 4428	61	0.0153	0.0219	-17%
15529	Metabolite - 3951	61	0.0125	0.019	-18%
11053	Metabolite - 2567	61	0.0053	0.0098	-18%
24213	Metabolite - 9845	61	0.0189	0.0261	-19%
20950	Metabolite - 7846	50	0.0915	0.0887	-19%
13065	Metabolite - 3146	61	0.0021	0.0045	-20%
1643	fumaric acid	50	0.0485	0.0558	-20%
1512	picolinic acid	50	0.0322	0.0396	-21%
24197	Metabolite - 9838	61	0.0675	0.0711	-22%
12724	Metabolite - 3057	61	0.0174	0.0241	-22%
27130	Metabolite - 10493	61	0.0087	0.0146	-22%
15676	3-methyl-2-oxovaleric acid	61	0.0716	0.0742	-23%
18871	Metabolite - 5848	61	0.0385	0.0468	-24%
13328	Metabolite - 3238	61	0.0194	0.0266	-24%
6253	Metabolite - 1283	61	0.0595	0.0644	-25%
10347	Metabolite - 2285	61	0.0015	0.0032	-25%

16712	Metabolite - 4432	61	0.0081	0.0139	-26%
14786	Metabolite - 3697	61	0.0795	0.0799	-27%
15683	4-methyl-2-oxopentanoate	61	0.0128	0.0191	-28%
8644	Metabolite - 2051	61	0.0413	0.0496	-28%
7650	Metabolite - 1834	61	7.29E-08	4.52E-07	-28%
6398	Metabolite - 1335	61	0.0617	0.0664	-29%
1412	2'-deoxyuridine	61	4.00E-04	0.001	-30%
19405	Metabolite - 6347	50	0.0877	0.0855	-31%
22130	DL-3-phenyllactic acid	61	0.0441	0.0522	-34%
26449	Metabolite - 10467	61	0.0777	0.079	-34%
25584	Metabolite - 10425	50	0.0078	0.0136	-34%
18929	Metabolite - 5907	50	0.0253	0.032	-35%
28059	Metabolite - 10650	50	1.00E-04	5.00E-04	-36%
5466	Metabolite - 1030	61	0.0098	0.016	-37%
20169	Metabolite - 7092	61	0.0582	0.0633	-37%
25459	Metabolite - 10395	50	0.0542	0.0604	-38%
17091	Metabolite - 4641	61	8.22E-07	4.50E-06	-38%
19968	Metabolite - 6930	50	0.0318	0.0395	-39%
11299	Metabolite - 2706	61	6.83E-08	4.38E-07	-39%
25505	Metabolite - 10402	61	4.00E-04	0.0012	-39%
16653	Metabolite - 4361	50	0.0032	0.0063	-41%
18968	Metabolite - 5919	61	4.00E-04	0.0011	-41%
11292	Metabolite - 2703	61	0.0059	0.0107	-41%
13534	Metabolite - 3320	61	0.0055	0.01	-43%
6297	Metabolite - 1304	61	0.0334	0.0409	-43%
19367	Metabolite - 6266	50	0.0035	0.0068	-44%
16071	Metabolite - 4020	50	1.00E-04	4.00E-04	-45%
24206	Metabolite - 9842	61	0.0142	0.0204	-47%
25602	Metabolite - 10432	50	8.00E-04	0.0018	-52%
25598	Metabolite - 10428	50	0.0665	0.0711	-58%
25597	Metabolite - 10427	50	0.0028	0.0056	-58%
25599	Metabolite - 10429	50	0.0319	0.0395	-59%
22548	Metabolite - 9026	50	0.0202	0.0274	-61%
19362	Metabolite - 6226	50	0.0243	0.0312	-65%
16650	Metabolite - 4360	50	0.008	0.0138	-66%
25538	Metabolite - 10415	61	0.0495	0.0562	-66%
25546	Metabolite - 10418	61	0.0018	0.0038	-73%
25527	Metabolite - 10410	61	2.00E-04	5.00E-04	-78%

22566	Metabolite - 9029	61	2.00E-04	6.00E-04	-80%
25529	Metabolite - 10411	61	3.00E-04	0.001	-81%
25517	Metabolite - 10406	61	6.00E-04	0.0016	-83%
10148	Metabolite - 2257	61	1.00E-04	5.00E-04	-84%
21651	Metabolite - 8410	61	6.66E-06	3.10E-05	-87%
25541	Metabolite - 10417	61	2.84E-05	1.00E-04	-91%
25539	Metabolite - 10416	61	3.59E-06	1.81E-05	-93%

[1] Isobar-71 includes conduritol-beta-epoxide-3-deoxyglucosone
[2] Isobar-56 includes DL-pipecolic acid-1-amino-1-cyclopentanecarboxylic acid
[3] Isobar-59 includes N-6-trimethyl-L-lysine-H-homoarg-OH
[4] Isobar-21-includes gamma-aminobutyryl-L-histidine-L-anserine
[5] Isobar-6 includes valine-betaine
[6] Isobar-1 includes mannose, fructose, glucose, galactose, alpha-L-sorbopyranose, Inositol, D-allose, D-altrose, D-psicone, L-gulose, allo-inositol

Table 15. Metabolite biomarkers of Atherosclerosis in aorta.

COMP_ ID	COMPOUND	LIB_ID	p-value	q-value	% Change in LDb
63	cholesterol	50	7.16E-07	8.78E-06	569%
22993	Metabolite - 9448	50	1.06E-05	1.00E-04	565%
10655	Metabolite - 2388	61	2.34E-07	4.83E-06	287%
25548	Metabolite - 10419	50	1.00E-04	3.00E-04	277%
22320	Metabolite - 8889	50	0.0015	0.0018	244%
15991	L-alpha-glycerophosphorylcholine	61	0.0174	0.0135	227%
27256	Metabolite - 10500	50	6.36E-07	8.78E-06	190%
19383	Metabolite - 6286	50	6.00E-04	0.001	125%
9137	Metabolite - 2141	61	0.0026	0.0028	121%
16028	Metabolite - 3998	50	0.0012	0.0015	110%
10739	Metabolite - 2407	61	0.0057	0.0053	94%
17987	Metabolite - 5228	50	0.0087	0.0078	92%
22675	Metabolite - 9126	61	9.00E-04	0.0013	89%
1481	inositol-1-phosphate	50	0.0149	0.0117	78%
30173	Metabolite - 10701	61	0.002	0.0022	70%
12774	Metabolite - 3094	50	0.0252	0.0186	66%
21421	Metabolite - 8214	50	0.0482	0.0321	63%
22032	Metabolite - 8766	50	0.0017	0.0019	62%
12604	Metabolite - 2981	50	0.0027	0.0028	54%
16002	Metabolite - 3992 [1]	61	1.71E-05	1.00E-04	37%

20360	Metabolite - 7326	61	0.0352	0.0243	-14%
7601	Metabolite - 1819	61	0.0351	0.0243	-14%
18829	phenylalanine	61	0.0303	0.0216	-16%
1604	uric acid	50	0.0331	0.0234	-17%
13505	Metabolite - 3313	61	0.0337	0.0237	-17%
19787	Metabolite - 6746	61	0.0143	0.0115	-18%
16235	Isobar-19 [2]	61	0.0433	0.0292	-18%
10743	Isobar-4 [3]	61	0.0428	0.0292	-18%
54	tryptophan	61	0.0217	0.0164	-19%
3147	xanthine	61	0.0234	0.0175	-19%
27570	Metabolite - 10569	61	0.0177	0.0135	-20%
15529	Metabolite - 3951	61	0.0468	0.0313	-20%
1125	isoleucine	50	0.0109	0.0092	-22%
1648	serine	50	0.0071	0.0065	-23%
605	uracil	50	0.0416	0.0286	-23%
7029	Metabolite - 1597	61	0.0045	0.0043	-24%
1299	tyrosine	61	0.008	0.0073	-24%
1592	N-acetylneuraminic acid	61	0.0087	0.0078	-24%
1508	pantothenic acid	61	0.0105	0.0089	-24%
13810	Metabolite - 3379	61	0.0146	0.0116	-24%
24076	Metabolite - 9726	50	1.00E-04	3.00E-04	-26%
8336	Metabolite - 2005	61	0.0019	0.0021	-26%
22258	Isobar-58 [4]	61	0.0292	0.0211	-26%
60	leucine	50	0.0045	0.0043	-28%
30128	Metabolite - 10687	61	0.0114	0.0095	-28%
17048	Metabolite - 4617	61	0.0014	0.0017	-29%
17960	Metabolite - 5207	50	0.0015	0.0018	-29%
15677	3-methyl-L-histidine	61	5.00E-04	0.001	-30%
1431	(p-Hydroxyphenyl)lactic acid	50	0.001	0.0013	-30%
1649	valine	50	0.0039	0.004	-30%
1898	proline	50	0.0042	0.0042	-30%
1284	threonine	50	0.0047	0.0045	-31%
15948	S-adenosyl-l-homocysteine	61	0.0101	0.0088	-31%
26456	Metabolite - 10470	61	0.0134	0.011	-31%
1302	methionine	61	0.0161	0.0125	-31%
22185	n-acetyl-l-aspartic acid	61	9.00E-04	0.0013	-32%
1414	3-phospho-d-glycerate	61	0.002	0.0022	-32%
1643	fumaric acid	50	0.0042	0.0042	-33%

10890	Metabolite - 2554	61	0.0029	0.003	-34%
10746	Isobar-6[5]	61	0.0283	0.0208	-35%
15253	Metabolite - 3832-possible-phenol-sulfate	61	0.0292	0.0211	-35%
5821	3-phospho-l-serine	61	7.00E-04	0.0012	-36%
12102	o-phosphoethanolamine	50	0.0014	0.0018	-36%
8404	Metabolite - 2027	61	0.0019	0.0021	-36%
1123	inosine	61	2.00E-04	5.00E-04	-37%
3127	hypoxanthine	61	8.00E-04	0.0012	-37%
6771	Metabolite - 1460	61	0.0015	0.0018	-37%
14311	Metabolite - 3481	61	0.0198	0.015	-37%
16233	Isobar-13 [6]	61	6.00E-04	0.001	-38%
11222	Metabolite - 2688	61	5.00E-04	0.001	-38%
1638	arginine	61	8.00E-04	0.0012	-38%
15497	arginino-succinate	61	0.0012	0.0015	-38%
15506	choline	61	2.00E-04	5.00E-04	-39%
16705	Metabolite - 4428	61	0.0012	0.0016	-39%
8991	Metabolite - 2105	61	0.0096	0.0085	-39%
514	cytidine	61	5.00E-04	9.00E-04	-40%
13018	Metabolite - 3138	61	5.00E-04	0.001	-40%
527	lactate	50	7.00E-04	0.0012	-40%
23051	Metabolite - 9566	61	0.001	0.0014	-40%
28131	Metabolite - 10670	61	4.00E-04	8.00E-04	-41%
1494	5-oxoproline	50	9.00E-04	0.0013	-41%
606	uridine	61	5.00E-04	0.001	-42%
18374	methionine-sulfoxide	61	1.00E-04	4.00E-04	-43%
57	glutamic acid	50	6.00E-04	0.001	-44%
15996	aspartate	50	8.00E-04	0.0012	-44%
20489	D-glucose	50	0.0051	0.0048	-44%
18348	3-hydroxy-3methylglutaryl-coenzyme-A	61	0.01	0.0087	-44%
27718	creatine	61	2.00E-04	5.00E-04	-45%
1303	malic acid	61	7.00E-04	0.0011	-45%
1412	2'-deoxyuridine	61	1.02E-06	1.09E-05	-46%
21430	Metabolite - 8266	61	2.00E-04	6.00E-04	-47%
11544	Metabolite - 2766	61	0.0013	0.0016	-47%
19372	Metabolite - 6269	50	0.0027	0.0028	-47%
22145	acetyl-L-carnitine	61	2.00E-04	5.00E-04	-48%

10737	Isobar-1[7]	61	3.00E-04	6.00E-04	-48%
14247	Metabolite - 3475	61	0.0146	0.0116	-48%
19110	Metabolite - 5978	50	3.00E-04	7.00E-04	-49%
22730	Metabolite - 9186	61	8.00E-04	0.0012	-49%
11777	glycine	50	5.00E-04	0.001	-50%
16228	Isobar-22[8]	61	1.00E-04	3.00E-04	-51%
16843	Metabolite - 4510	50	1.00E-04	3.00E-04	-51%
53	glutamine	50	2.00E-04	6.00E-04	-51%
19708	Metabolite - 6711	61	0.0124	0.0103	-51%
1416	GABA	50	1.38E-08	1.18E-06	-52%
15500	carnitine	61	5.00E-04	9.00E-04	-52%
2125	taurine	61	0.0017	0.002	-52%
22475	Metabolite - 8986	61	0.0044	0.0043	-52%
28059	Metabolite - 10650	50	1.87E-05	1.00E-04	-54%
20361	Metabolite - 7327	61	3.32E-05	2.00E-04	-54%
17971	Metabolite - 5210	50	1.00E-04	3.00E-04	-54%
22494	Metabolite - 8994	50	4.00E-04	9.00E-04	-54%
7650	Metabolite - 1834	61	2.06E-07	4.83E-06	-55%
27738	threonic acid	50	1.00E-04	3.00E-04	-55%
27678	Metabolite - 10584	50	3.00E-04	7.00E-04	-55%
12459	Isobar-10[9]	61	3.43E-05	2.00E-04	-56%
15125	(2-Aminoethyl)phosphonate	61	1.00E-04	3.00E-04	-56%
19934	inositol	50	3.00E-04	7.00E-04	-56%
16860	Metabolite - 4517	50	1.00E-04	3.00E-04	-57%
17064	Metabolite - 4624	50	1.00E-04	4.00E-04	-57%
1107	allantoin	50	5.00E-04	0.001	-57%
1573	guanosine	61	4.00E-06	3.69E-05	-58%
1670	urea	50	1.00E-04	3.00E-04	-58%
27727	glutathione-oxidized	61	1.00E-04	4.00E-04	-61%
1519	sucrose	50	0.0104	0.0089	-62%
22702	Metabolite - 9127	61	4.30E-06	3.69E-05	-63%
15336	tartaric acid	61	6.92E-08	2.97E-06	-66%
6172	Metabolite - 1245	61	2.82E-07	4.83E-06	-70%
17975	Metabolite - 5211	50	1.04E-05	1.00E-04	-70%
27773	Isobar-71[10]	61	3.00E-04	7.00E-04	-71%

[1] Possibly Cl-adduct of Formate dimmer

[2] Isobar-19 includes 1,5-anhydro-D-glucitol, 2'-deoxy-D-galactose, 2'-deoxy-D-glucose, L-fucose, L-rhamnose

[3] Isobar-4 includes Gluconic acid, DL-arabinose, D-ribose, L-xylose, DL-lyxose, D-xylulose, galactonic acid
[4] Isobar-58 includes bicine, 2-methylaminomethyl-tartronic acid
[5] Isobar-6 includes valine-betaine
[6] Isobar-13 includes 5-keto-D-gluconic acid, 2-keto-L-gulonic acid, D-glucuronic acid, D-galacturonic acid
[7] Isobar-1 includes mannose, fructose, glucose, galactose, alpha-L-sorbopyranose, Inositol, D-allose, D-altrose, D-psicone, L-gulose, allo-inositol
[8] Isobar-22-includes-glutamic acid-O-acetyl-L-serine
[9] Isobar-10-includes-glutamine-H-beta-ala-gly-OH-1-methylguanine-H-Gly-Sar-OH-lysine
[10] Isobar-71includes conduritol-beta-epoxide-3-deoxyglucosone

Table 16. Metabolite biomarkers of Atherosclerosis in liver.

COMP_ ID	COMPOUND	LIB_ID	p-value	q-value	% Change in LDb
19788	Metabolite - 6747	61	6.49E-06	0.001	895%
1564	citric acid	50	0.0069	0.0454	399%
9117	Metabolite - 2135	61	0.0173	0.0851	206%
25626	Metabolite - 10443	61	0.021	0.0964	180%
19597	Metabolite - 6648	50	0.0055	0.0452	162%
15685	5-hydroxylysine	61	0.0049	0.0452	159%
16655	Metabolite - 4362	50	0.0064	0.0454	131%
21418	Isobar-56[1]	61	1.36E-05	0.0011	129%
22475	Metabolite - 8986	61	0.0012	0.0191	129%
15803	maltose	50	0.0224	0.0992	128%
18344	D-xyulose	50	1.00E-04	0.0071	126%
27678	Metabolite - 10584	50	0.0083	0.049	126%
22020	Metabolite - 8749	50	6.00E-04	0.0168	124%
30204	Metabolite - 10713	61	0.0138	0.0703	122%
30203	Metabolite - 10712	61	9.00E-04	0.0186	113%
15053	sorbitol	50	0.0048	0.0452	105%
1640	ascorbic acid	50	0.0032	0.0375	104%
19753	Metabolite - 6718	61	0.0073	0.046	97%
21650	Metabolite - 8409	61	0.0185	0.0866	91%
1516	sarcosine	50	0.0124	0.0659	85%
22309	Metabolite - 8887	61	0.001	0.0191	85%
27299	Metabolite - 10520	61	4.00E-04	0.0136	83%
1118	eicosanoic acid	50	0.0015	0.0191	78%
25429	Metabolite - 10369	50	0.0045	0.0452	72%
8669	Metabolite - 2055	61	0.0087	0.0498	69%
8210	Metabolite - 1981	61	0.023	0.0992	65%

20488	D-glucose	50	0.0073	0.046	55%
15606	Metabolite - 3968	61	0.0069	0.0454	51%
11379	Metabolite - 2725	61	0.0224	0.0992	50%
11484	Metabolite - 2752	61	7.00E-04	0.0168	50%
11292	Metabolite - 2703	61	0.0133	0.0694	49%
8457	Metabolite - 2035 [2]	61	0.0066	0.0454	47%
16859	Metabolite - 4516	50	0.0066	0.0454	44%
554	adenine	50	0.0076	0.0463	44%
7081	Metabolite - 1609	61	0.0039	0.0412	41%
20795	Metabolite - 7747	61	0.0033	0.0375	40%
16229	Isobar-24 [3]	61	0.0055	0.0452	39%
18388	Metabolite - 5491	50	0.0057	0.0452	39%
24360	Metabolite - 10206	50	0.0178	0.086	38%
12080	D-ribose	50	0.0104	0.0575	37%
22993	Metabolite - 9448	50	0.0156	0.0782	36%
10737	Isobar-1 [4]	61	0.0084	0.049	36%
16060	Metabolite - 4014	50	0.0181	0.0862	36%
24285	Metabolite - 10026	61	0.0229	0.0992	23%
63	cholesterol	50	0.0104	0.0575	15%
22414	Metabolite - 8933	61	0.0037	0.0406	-29%
1827	riboflavine	61	0.0052	0.0452	-29%
22320	Metabolite - 8889	50	0.0056	0.0452	-29%
9002	Metabolite - 2107	61	0.0012	0.0191	-31%
3138	pyridoxamine-phosphate	61	0.0054	0.0452	-34%
15964	D-arabitol	50	2.00E-04	0.0098	-34%
7432	Metabolite - 1735	61	0.0014	0.0191	-35%
22185	n-acetyl-l-aspartic acid	61	0.0068	0.0454	-36%
23024	Metabolite - 9458	61	0.0011	0.0191	-37%
21296	glucosamine-6-sulfate	61	0.0014	0.0191	-39%
7650	Metabolite - 1834	61	7.40E-06	0.001	-41%
9468	Metabolite - 2183	61	8.00E-04	0.0182	-46%
6530	Metabolite - 1384	61	0.0115	0.0621	-57%
25561	Metabolite - 10421	61	0.0069	0.0454	-58%
597	phosphoenolpyruvate	61	0.0014	0.0191	-59%
1414	3-phospho-d-glycerate	50	1.57E-05	0.0011	-60%
10148	Metabolite - 2257	61	0.003	0.037	-68%
27794	Metabolite - 10587	61	4.00E-04	0.0127	-69%
6146	alpha-amino-adipate	50	2.00E-04	0.0098	-73%

[1] Isobar-56 includes DL-pipecolic acid-1-amino-1-cyclopentanecarboxylic acid
[2] Possible 5-methyl-deoxycytidine-monophosphate
[3] Isobar-24 includes L-arabitol, adonitol, xylitol
[4] Isobar-1 includes mannose, fructose, glucose, galactose, alpha-L-sorbopyranose, Inositol, D-allose, D-altrose, D-psicone, L-gulose, allo-inositol

[00197] Identification of plasma biomarkers indicative of initiation and/or progression of atherosclerosis would help diagnosis and treatment of human patients with this disease. Recursive partitioning of plasma metabolites identified cholesterol as a biomarker that could differentiate the LDb and C57BL/6 mice perfectly, as expected. Three other metabolites were also identified by recursive partitioning to differentiate the LDb and C57BL/6 mice (Table 17). Plasma levels of these metabolites, like plasma cholesterol, were higher in LDb mice even at 2 months of age and remained consistently higher during the following 6 months, suggesting that earlier buildup of these metabolites is likely involved in the development of atherosclerosis and provide biomarkers for progression (Figures 13, 14, 15, and 16).

Table 17. Atherosclerosis biomarkers in plasma that differentiate Atherosclerosis subjects (LDb) and healthy control subjects (C57BL/6 mice) without error.

Cmpd_ID	Compound	Atherosclerosis level	Control level	R-Square	LogWorth
19323	Docosahexaenoic-Acid	≥528666	<528666	1.000	21.11
21011	Metabolite - 7888	≥2523794	<2523794	1.000	21.11
21631	Metabolite - 8403	≥238349	<238349	1.000	21.11

[00198] Metabolite – 1834 did not segregate LDb and C57BL/6 groups in 2-month-old mice, but started to segregate the 5-month-olds and segregated 8-month-old mice perfectly (Figure 17). This metabolite is one of the biomarkers for atherosclerosis progression.

[00199] Several metabolites (p-cresol-sulfate, Metabolite - 4887, Metabolite - 5386) classified subjects as LDb or C57BL/6 very well in 2-month-old mice, but the power of differentiation diminished as the mice aged, with no segregation in 8-month-old mice (Figures 18 and 19). These metabolites are biomarkers for atherosclerosis initiation.

[00200] Random forest results show that the samples can be classified correctly with varying degrees of accuracy using the biomarkers. The confusion matrices demonstrate that LDb subjects can be distinguished from C57BL/6 subjects using plasma (Table 18), aorta (Table 19) and liver (Table 20) samples. The "Out-of-Bag" (OOB) Error rate gives an estimate of how accurately new observations can be predicted using the random forest model (e.g., whether a sample is from a subject having atherosclerosis or a control subject).

Table 18. Random Forest Confusion Matrices for Atherosclerosis in Plasma

Plasma			
Age Collected:	**2 months**		
	Control	**Atherosclerosis**	**Error**
Control	10	0	0
Atherosclerosis	0	10	0
OOB Error	0	**0%**	
Age Collected:	**5 months**		
	Control	**Atherosclerosis**	**Error**
Control	10	0	0
Atherosclerosis	2	9	0
OOB Error	0	**0%**	
Age Collected:	**8 months**		
	Control	**Atherosclerosis**	**Error**
Control	8	0	0
Atherosclerosis	0	8	0
OOB Error	0	**0%**	
ALL			
	Control	**Atherosclerosis**	**Error**
Control	28	0	0
Atherosclerosis	0	27	0
OOB Error	0	**0%**	

Table 19. Random Forest Confusion Matrices for Atherosclerosis in Aorta Tissues

Aorta Tissue			
Age Collected:	**2 months**		
	Control	**Atherosclerosis**	**Error**
Control	6	1	0.14
Atherosclerosis	2	2	0.5
OOB Error	3/11=0.27	**27%**	
Age Collected:	**5 months**		
	Control	**Atherosclerosis**	**Error**
Control	7	0	0
Atherosclerosis	2	4	0.33
OOB Error	2/13=0.15	**15%**	
Age Collected:	**8 months**		
	Control	**Atherosclerosis**	**Error**
Control	6	0	0
Atherosclerosis	0	4	0
OOB Error	0/10=0.00	**0%**	
ALL			
	Control	**Atherosclerosis**	**Error**
Control	18	2	0.1
Atherosclerosis	3	11	0.21
OOB Error	5/34=0.15	**15%**	

Table 20. Random Forest Confusion Matrices for Atherosclerosis in Liver

Liver			
Age Collected:	2 months		
	Control	Atherosclerosis	Error
Control	7	1	0.13
Atherosclerosis	1	3	0.25
OOB Error	2/12=0.17	17%	
Age Collected:	5 months		
	Control	Atherosclerosis	Error
Control	3	1	0.25
Atherosclerosis	3	3	0.5
OOB Error	4/10=0.4	40%	
Age Collected:	8 months		
	Control	Atherosclerosis	Error
Control	5	0	0
Atherosclerosis	1	5	0.17
OOB Error	1/11=0.09	9%	
ALL			
	Control	Atherosclerosis	Error
Control	15	2	0.12
Atherosclerosis	3	13	0.19
OOB Error	5/33=0.15	15%	

[00201] In addition, a study was carried out on human subjects suffering from atherosclerosis (n=15) or healthy subjects (n=14). The biomarkers 3-methylhistidine, p-cresol sulfate, mannose, glucose, and gluconate showed the same alterations in human plasma from disease vs. healthy subjects as seen in the mouse model. Thus, these compounds were identified as important biomarkers useful to distinguish individuals with atherosclerosis from healthy subjects.

3C: Biomarkers of cardiomyopathy

[00202] Biomarkers were discovered by (1) analyzing cardiac tissue samples (Table 21) or plasma samples (Table 22) from different groups of mouse subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the two groups. These subjects provide an animal (mouse) model for human DCM.

[00203] Two groups of subjects were used. One group consisted of eight subjects

exhibiting cardiac dilatation and depressed left ventricular systolic function (ejection fraction of less than 0.40), as determined by echocardiography (cTnT-W141 transgenic mice). Thirteen age- and gender-matched subjects (non-transgenic (wild-type background strain) mice) served as controls. All mice were 7-19 months old and weighed 23-40 gm.

[00204] T-tests were used to determine differences in the mean levels of metabolites between the two populations (i.e., Dilated Cardiomyopathy, DCM vs. Healthy control). Classification analysis was carried out using recursive partitioning and random forest analyses to uncover the biomarkers that can best differentiate the two groups. Recursive partitioning relates a 'dependent' variable (Y) to a collection of independent ('predictor') variables (X) in order to uncover -- or simply understand -- the elusive relationship, Y=f(X). It was performed with the JMP program (SAS) to generate a decision tree. The statistical significance of the "split" of the data can be placed on a more quantitative footing by computing p-values, which discern the quality of a split relative to a random event. The significance level of each "split" of data into the nodes or branches of the tree was computed as p-values, which discern the quality of the split relative to a random event. It was given as LogWorth, which is the negative log10 of a raw p-value.

Biomarkers:

[00205] As listed below in Tables 21 and 22, biomarkers were discovered that were differentially present between cardiac tissue and plasma samples, respectively, collected from dilated cardiomyopathy subjects and healthy subjects.

[00206] Tables 21 and 22 include, for each listed biomarker, the p-value and q-value determined in the statistical analysis of the data concerning the biomarkers and an indication of the percentage difference in the dilated cardiomyopathy mean level as compared to the healthy mean level in cardiac tissue (Table 21) or plasma (Table 22). The term "Isobar" as used in the tables indicates the compounds that could not be distinguished from each other on the analytical platform used in the analysis (i.e., the compounds in an isobar elute at nearly the same time and have similar (and sometimes exactly the same) quant ions, and thus cannot be distinguished). Comp_ID refers to the compound identification number used as a primary key for that compound in the in-house chemical database. Library indicates the chemical library that was used to identify the

compounds. The number 50 refer to the GC library and the number 61 refers to the LC library.

Table 21. Metabolite biomarkers of dilated cardiomyopathy (DCM) in cardiac tissues.

COMP_ ID	COMPOUND	LIB_ ID	p-value	q-value	% Change in DCM
22185	n-acetyl-l-aspartic acid	61	0.0044	0.0144	100%
15996	aspartate	50	0.0002	0.0022	64%
1414	3-phospho-d-glycerate	50	0.0742	0.0888	52%
1898	proline	61	3.64E-05	0.0007	52%
1648	serine	50	0.0009	0.0057	49%
1299	tyrosine	61	3.36E-05	0.0007	39%
1284	threonine	50	0.0054	0.0173	28%
54	tryptophan	61	0.0383	0.0571	20%
1649	valine	50	0.0081	0.0227	19%
1125	isoleucine	50	0.0041	0.014	16%
11777	glycine	50	0.0109	0.0255	15%
13179	creatine	61	0.0011	0.0061	-17%
590	hypotaurine	61	0.0728	0.0879	-21%
5278	beta-nicotinamide adenine dinucleotide	61	0.0092	0.0239	-27%
15500	carnitine	61	0.0331	0.0509	-32%
2127	glutathione, reduced	61	0.0033	0.0125	-62%
12080	D-ribose	50	0.0033	0.0125	39%
15122	glycerol	50	0.0663	0.0819	-16%
19934	inositol	50	0.0225	0.0417	-22%
18882	taurodeoxycholic acid	61	0.0584	0.0754	72%
15365	*sn*-Glycerol 3-phosphate	50	0.0109	0.0255	41%
63	cholesterol	50	0.011	0.0255	14%
1121	heptadecanoic acid	50	0.0594	0.0761	-15%
21127	monopalmitin	50	0.0149	0.0324	-23%
1336	n-hexadecanoic acid	50	0.0014	0.0072	-24%
19323	docosahexaenoic acid	50	0.0009	0.0057	-24%
1600	o-phosphoethanolamine	50	0.0253	0.0435	-30%
1365	tetradecanoic acid	50	0.0516	0.0691	-31%
1570	oleic acid	50	0.0269	0.0446	-31%

1105	linoleic acid	50	0.0014	0.0072	-32%
1518	squalene	50	0.0013	0.0072	-43%
15504	phosphopantheine	61	0.0186	0.0367	-49%
1827	riboflavine	61	0.0037	0.0129	85%
594	niacinamide	50	0.0091	0.0239	-17%
3138	pyridoxamine phosphate	61	0.0047	0.0153	-19%
1508	pantothenic acid	61	0.0093	0.0239	-32%
3127	hypoxanthine	50	0.0112	0.0255	92%
606	uridine	61	0.0262	0.0445	56%
1107	allantoin	50	0.0207	0.0398	39%
514	cytidine	61	0.0034	0.0126	33%
1573	guanosine	61	0.0326	0.0507	32%
605	uracil	50	0.0291	0.0457	27%
21031	hydroxyurea	61	0.0069	0.0204	-47%
2856	uridine 5'-monophosphate	61	0.0005	0.004	-68%
18360	adenylosuccinic acid	61	9.02E-06	0.0004	-85%
555	adenosine	61	0.0007	0.0051	-87%
2832	adenosine 5'-monophosphate	61	4.53E-06	0.0004	-90%
2849	guanosine 5'-monophosphate	61	2.06E-05	0.0006	-94%
20701	malitol	50	0.0167	0.0343	-32%
8469	Metabolite - 2036	61	0.0144	0.0319	525%
10781	Metabolite - 2469	61	0.007	0.0204	355%
10604	Metabolite - 2370	61	0.0089	0.0239	213%
10401	Metabolite - 2058	61	0.0006	0.0048	203%
5597	Metabolite - 1073	61	0.0178	0.0357	203%
14639	Metabolite - 3603	61	5.89E-05	0.001	194%
16019	Metabolite - 3995	61	0.0001	0.0017	156%
22480	Metabolite - 8987	50	1.78E-05	0.0006	150%
6130	Metabolite - 1208	61	0.0277	0.0454	144%
9137	Metabolite - 2141	61	0.0174	0.0353	127%
21418	Isobar-56[1]	61	0.0015	0.0072	122%
18015	Metabolite - 3113	61	0.0015	0.0072	122%
22414	Metabolite - 8933	61	0.0003	0.0028	108%
6122	Metabolite - 1206[2]	61	0.0414	0.058	104%
9024	Metabolite - 2111	61	0.0241	0.0432	104%
18073	Metabolite - 5270	61	0.0159	0.0337	92%
12711	Metabolite - 3053	61	0.0574	0.0752	89%
13512	Metabolite - 3315	61	0.028	0.0454	89%

16471	Metabolite - 4238	61	0.046	0.0627	89%
5618	Metabolite - 1085[3]	61	0.0371	0.0558	85%
7654	Metabolite - 1836	61	0.002	0.0085	82%
16860	Metabolite - 4517	50	0.0027	0.011	79%
14715	Metabolite - 3653[4]	61	0.0037	0.0129	72%
21410	Isobar-52[5]	61	0.0018	0.008	69%
17885	Metabolite - 5147	61	0.0155	0.0332	56%
22494	Metabolite - 8994	50	0.0003	0.003	56%
10850	Metabolite - 2548[6]	61	0.0668	0.0819	52%
15213	Metabolite - 3808	61	5.92E-06	0.0004	52%
21415	Metabolite - 8209	50	0.011	0.0255	52%
6266	Metabolite - 1286	61	9.64E-05	0.0013	49%
7127	Metabolite - 1616	61	0.0019	0.0084	49%
7272	Metabolite - 1679	61	0.0562	0.0746	45%
8509	Metabolite - 2041	61	0.0104	0.0255	45%
9313	Metabolite - 2172	61	0.0246	0.0432	45%
9905	Metabolite - 2231	61	0.0167	0.0343	43%
16071	Metabolite - 4020	50	0.002	0.0085	41%
22441	Metabolite - 8950	61	0.0798	0.0925	39%
19273	Metabolite - 6108	61	0.0405	0.058	37%
16233	Isobar-13[7]	61	0.0126	0.0282	33%
9324	Metabolite - 2173	61	0.0577	0.0752	33%
19787	Metabolite - 6746	61	0.0244	0.0432	30%
20299	Metabolite - 7266	50	0.0212	0.0402	30%
9122	Metabolite - 2137	61	0.0836	0.0961	28%
21404	Isobar-48[8]	61	0.0487	0.0658	27%
21011	Metabolite - 7888	50	0.0031	0.012	27%
13142	Metabolite - 3165	61	0.0412	0.058	18%
19372	Metabolite - 6269	50	0.0192	0.0374	16%
16285	Metabolite - 2798	50	0.045	0.062	15%
13505	Metabolite - 3313	61	0.0338	0.0514	11%
17064	Metabolite - 4624	50	0.0619	0.0779	-15%
19599	Metabolite - 6649	50	0.0287	0.0457	-18%
16074	Metabolite - 2758	50	0.0417	0.058	-19%
20361	Metabolite - 7327	61	0.0008	0.0056	-20%
7081	Metabolite - 1609	61	0.0604	0.0767	-21%
17919	Metabolite - 5187	61	0.0072	0.0208	-22%
17978	Metabolite - 5213	50	0.0689	0.0838	-23%

18273	Metabolite - 5420	50	0.0784	0.0916	-23%
16984	Metabolite - 4599	50	0.0624	0.0779	-24%
11545	Metabolite - 2767	61	0.0412	0.058	-25%
22509	Metabolite - 9011	61	0.0063	0.0191	-29%
12856	Metabolite - 3123	61	0.0768	0.0904	-32%
16060	Metabolite - 4014	50	0.0253	0.0435	-32%
16843	Metabolite - 4510	50	0.0289	0.0457	-34%
17960	Metabolite - 5207	50	0.0003	0.003	-35%
16116	Metabolite - 4051	50	0.0226	0.0417	-36%
14595	Metabolite - 3576	61	0.0106	0.0255	-36%
8176	Metabolite - 1974	61	0.0008	0.0056	-37%
15085	Metabolite - 3776	61	0.0093	0.0239	-39%
16705	Metabolite - 4428	61	0.0059	0.0182	-40%
19505	Metabolite - 6547	61	0.0267	0.0446	-41%
11056	Metabolite - 2568	61	0.075	0.0891	-43%
22507	Metabolite - 9010	50	0.0396	0.0578	-46%
9130	Metabolite - 2139	61	0.001	0.006	-48%
22381	Metabolite - 8908	61	0.0389	0.0574	-50%
18702	Metabolite - 5767	61	0.0234	0.0428	-56%
11379	Metabolite - 2725	61	6.71E-05	0.001	-69%
22501	Metabolite - 9007	61	0.0007	0.0052	-79%
22534	Metabolite - 9016	61	2.36E-05	0.0006	-86%

[1] Isobar-56 includes DL-pipecolic acid,1-amino-1-cyclopentanecarboxylic acid.

[2] Possible methyltestosterone and others.

[3] Possible isolobinine or 4-aminoestra-1,3,5(10)-triene-3,17beta-diol.

[4] Possible stachydrine.

[5] Isobar-52 includes iminodiacetic acid, L-aspartic acid.

[6] Possible Cl adduct of uric acid.

[7] Isobar 13 includes 5-keto-D-gluconic acid, 2-keto-L-gulonic acid, D-glucuronic acid,D(+)-galacturonic acid.

[8] Isobar 48 includes Serine-2,2-amino-2-methyl-1,3-propanediol,diethanolamine.

Table 22. Metabolite biomarkers of dilated cardiomyopathy (DCM) in plasma.

COMP_ ID	COMPOUND	LIB_ ID	p-value	q-value	% Change in DCM	TG/NTG
17007	Metabolite - 4609	61	0.0003	0.0895	83%	1.83
1299	tyrosine	61	0.0005	0.0895	83%	1.83
20161	Metabolite - 7088	61	0.0008	0.0895	239%	3.39
19787	Metabolite - 6746	61	0.0010	0.0895	62%	1.62
20699	meso-erythritol	50	0.0013	0.0895	46%	1.46
18968	Metabolite - 5919	61	0.0014	0.0895	100%	2.00
1107	allantoin	50	0.0017	0.0901	54%	1.54
1431	(p-Hydroxyphenyl)lactic acid	61	0.0028	0.1283	114%	2.14

584	mannose	50	0.0034	0.1411	-41%	0.59
15632	Metabolite - 3980	61	0.0054	0.1997	64%	1.64
396	glutarate	61	0.0069	0.2328	181%	2.81
11292	Metabolite - 2703	61	0.0080	0.2473	124%	2.24
18829	phenylalanine	61	0.0098	0.2547	39%	1.39
15286	Metabolite - 3848	61	0.0103	0.2547	92%	1.92
13575	Metabolite - 3324	61	0.0112	0.2547	250%	3.50
14786	Metabolite - 3697	61	0.0121	0.2547	52%	1.52
22597	Metabolite - 9041	61	0.0130	0.2547	-70%	0.30
15611	Metabolite - 3971	61	0.0132	0.2547	66%	1.66
11813	Metabolite - 2809	61	0.0137	0.2547	-28%	0.72
6571	Metabolite - 1397	61	0.0139	0.2547	75%	1.75
21418	Isobar 56 includes DL-pipecolic acid,1-amino-1-cyclopentanecarboxylic acid	61	0.0151	0.2547	38%	1.38
11299	Metabolite - 2706	61	0.0159	0.2547	65%	1.65
6305	Metabolite - A-1254	61	0.0163	0.2547	64%	1.64
19857	Metabolite - 6783	61	0.0165	0.2547	135%	2.35
21044	(s)-2-hydroxybutyric acid	50	0.0186	0.2722	105%	2.05
16016	Metabolite - 3994	61	0.0209	0.2722	92%	1.92
5440	Metabolite - A-1014	61	0.0221	0.2722	41%	1.41
22555	Metabolite - 9027	50	0.0223	0.2722	-53%	0.47
10737	Isobar 1 includes mannose, fructose, glucose, galactose, alpha-L-sorbopyranose, Inositol, D-allose,D-(+)-altrose,D-psicone,L-(+)-gulose, allo-inositol	61	0.0227	0.2722	-33%	0.67
20830	Metabolite - 7762	61	0.0231	0.2722	48%	1.48
14961	Metabolite - 3752	61	0.0234	0.2722	-46%	0.54
15670	2-methylhippuric acid	61	0.0234	0.2722	53%	1.53
13142	Metabolite - 3165	61	0.0285	0.3038	37%	1.37
605	uracil	50	0.0286	0.3038	217%	3.17
21011	Metabolite - 7888	50	0.0298	0.3038	37%	1.37
8959	Metabolite - 2100	61	0.0305	0.3038	-29%	0.71
20169	Metabolite - 7092	61	0.0320	0.3038	88%	1.88
5776	Metabolite - A-1194	61	0.0322	0.3038	89%	1.89
6126	Metabolite - 1207	61	0.0326	0.3038	52%	1.52
12459	Isobar 10 includes glutamine, H-beta-ala-gly-OH, 1-methylguanine, H-Gly-Sar-OH lysine	61	0.0327	0.3038	-31%	0.69
3155	3-ureidopropionic acid	61	0.0351	0.3093	60%	1.60
15541	Metabolite - 3957	61	0.0360	0.3093	43%	1.43
1303	malic acid	50	0.0388	0.3093	108%	2.08
15737	hydroxyacetic acid	50	0.0392	0.3093	38%	1.38
527	lactate	50	0.0399	0.3093	65%	1.65
1670	urea	50	0.0410	0.3093	23%	1.23
15949	2'-deoxycytidine	61	0.0413	0.3093	24%	1.24
7272	Metabolite - 1679	61	0.0415	0.3093	251%	3.51

12011	Metabolite - 2848	61	0.0425	0.3093	53%	1.53
1643	fumaric acid	50	0.0437	0.3093	70%	1.70
1574	histamine	61	0.0452	0.3093	74%	1.74
22566	Metabolite - 9029	61	0.0458	0.3093	-63%	0.37
15140	L-kynurenine	61	0.0458	0.3093	49%	1.49
22026	1-methylguanidine hydrochloride	50	0.0462	0.3093	19%	1.19
7127	Metabolite - 1616	61	0.0473	0.3093	-38%	0.62
1604	uric acid	50	0.0478	0.3093	146%	2.46
7429	Metabolite - 1733	61	0.0492	0.3093	37%	1.37
12626	Metabolite - 3003	50	0.0500	0.3093	51%	1.51
2849	guanosine 5'- monophosphate	61	0.0509	0.3093	-58%	0.42
16327	Metabolite - 4161	61	0.0514	0.3093	35%	1.35
14715	Metabolite - 3653 - Possible stachydrine	61	0.0521	0.3093	29%	1.29
1507	palmitoleic acid	50	0.0522	0.3093	-54%	0.46
9491	Metabolite - 2185	61	0.0525	0.3093	97%	1.97
2734	gamma-L-glutamyl-L-tyrosine	61	0.0541	0.3112	34%	1.34
11235	Metabolite - 2690	61	0.0548	0.3112	-34%	0.66
13775	Metabolite - 3370	61	0.0561	0.3112	38%	1.38
17960	Metabolite - 5207	50	0.0569	0.3112	-32%	0.68
1587	N-acetyl-L-leucine	61	0.0577	0.3112	75%	1.75
20798	Metabolite - 7748	61	0.0581	0.3112	-41%	0.59
2832	adenosine 5'-monophosphate	61	0.0589	0.3112	-80%	0.20
19294	Metabolite - 6134	61	0.0596	0.3112	31%	1.31
15278	Metabolite - 3843	61	0.0603	0.3112	-31%	0.69
15255	Metabolite - 3833	61	0.0614	0.3127	74%	1.74
16468	Metabolite - 4236	61	0.0644	0.3224	-31%	0.69
10309	Metabolite - 2277	61	0.0651	0.3224	35%	1.35
1302	methionine	61	0.0705	0.3425	21%	1.21
17885	Metabolite - 5147	61	0.0710	0.3425	76%	1.76
13038	Metabolite - 3143	61	0.0726	0.3440	53%	1.53
11411	Metabolite - 2746	61	0.0732	0.3440	79%	1.79
6373	Metabolite - A-1304	61	0.0749	0.3454	-25%	0.75
22259	Isobar 59 includes N('6)-trimethyl-L-lysine,H-homoarg-OH	61	0.0756	0.3454	-29%	0.71
7081	Metabolite - 1609	61	0.0762	0.3454	67%	1.67
2856	uridine 5'-monophosphate	61	0.0807	0.3484	-54%	0.46
16983	Metabolite - 4598	50	0.0813	0.3484	34%	1.34
20092	Metabolite - 7050	61	0.0818	0.3484	-22%	0.78
14439	Metabolite - 3498	61	0.0833	0.3484	26%	1.26
12682	Metabolite - 3044	61	0.0841	0.3484	77%	1.77
18281	2-hydroxyhippuric acid	61	0.0843	0.3484	21%	1.21
12129	beta-hydroxyisovaleric acid	50	0.0864	0.3484	17%	1.17
14117	Metabolite - 3441	61	0.0867	0.3484	-20%	0.80
20488	D-glucose	50	0.0874	0.3484	-25%	0.75
9216	Metabolite - 2168	61	0.0884	0.3484	-21%	0.79
19596	Metabolite - 6647	50	0.0885	0.3484	28%	1.28

16819	Metabolite - 4496	50	0.0909	0.3484	19%	1.19
22584	Metabolite - 9038	61	0.0910	0.3484	-55%	0.45
21650	Metabolite - 8409	61	0.0910	0.3484	-38%	0.62
22598	Metabolite - 9042	61	0.0920	0.3484	-65%	0.35
54	tryptophan	61	0.0927	0.3484	19%	1.19
16655	Metabolite - 4362	50	0.0946	0.3484	-37%	0.63
19402	Metabolite - 6346	50	0.0969	0.3484	-21%	0.79
11661	indole-3-pyruvic acid	61	0.0972	0.3484	60%	1.60
1561	alpha-tocopherol	50	0.0974	0.3484	38%	1.38
8180	Metabolite - 1975	61	0.0982	0.3484	35%	1.35
16666	Metabolite - 4365	50	0.0983	0.3484	-28%	0.72
2132	citrulline	50	0.0985	0.3484	51%	1.51
1508	pantothenic acid	61	0.1014	0.3489	37%	1.37
1572	glyceric acid	50	0.1017	0.3489	14%	1.14
21732	Metabolite - 8475	61	0.1020	0.3489	-38%	0.62
9130	Metabolite - 2139	61	0.1024	0.3489	105%	2.05
21654	Metabolite - 8413	61	0.1043	0.3522	-25%	0.75
10461	Metabolite - 2313	61	0.1057	0.3534	-27%	0.73
16511	Metabolite - 4274	50	0.1072	0.3534	69%	1.69
1638	arginine	50	0.1075	0.3534	128%	2.28
13345	Metabolite - 3244	61	0.1087	0.3543	24%	1.24
1493	ornithine	50	0.1183	0.3823	105%	2.05
5809	3-indoxyl sulfate	61	0.1251	0.3991	80%	1.80
1648	serine	50	0.1261	0.3991	73%	1.73
1826	folic acid	61	0.1268	0.3991	-66%	0.34
512	asparagine	50	0.1357	0.4181	64%	1.64
514	cytidine	61	0.1358	0.4181	69%	1.69
16244	Isobar 21 includes gamma-aminobutyryl-L-histidine, L-anserine	61	0.1365	0.4181	-31%	0.69
1598	N-tigloylglycine	61	0.1373	0.4181	16%	1.16
17091	Metabolite - 4641	61	0.1396	0.4217	40%	1.40
17665	p-hydroxybenzaldehyde	61	0.1424	0.4242	18%	1.18
1494	5-oxoproline	50	0.1430	0.4242	24%	1.24
8336	Metabolite - 2005	61	0.1439	0.4242	82%	1.82
13214	Metabolite - 3183 - possible gamma-L-glutamyl-L-phenylalanine	61	0.1516	0.4351	28%	1.28
1336	n-hexadecanoic acid	50	0.1518	0.4351	-14%	0.86
21701	Metabolite - 8454	61	0.1537	0.4351	-34%	0.66
10141	Metabolite - A-2035	61	0.1540	0.4351	-15%	0.85
17028	Metabolite - 4611	50	0.1542	0.4351	19%	1.19
1432	alphahydroxybenzeneacetic acid	61	0.1546	0.4351	-27%	0.73
6771	Metabolite - 1460	61	0.1581	0.4415	-25%	0.75
12774	Metabolite - 3094	50	0.1632	0.4513	-13%	0.87
20084	Metabolite - 7047	61	0.1656	0.4513	17%	1.17
1651	pyridoxal	61	0.1692	0.4513	24%	1.24
12924	Metabolite - 3131	61	0.1702	0.4513	20%	1.20
8072	Metabolite - 1958	61	0.1703	0.4513	17%	1.17

1126	alanine	50	0.1714	0.4513	61%	1.61
22567	Metabolite - 9030	61	0.1721	0.4513	-51%	0.49
6413	Metabolite - 1342 - possible phenylacetylglutamine or formyl-N-acetyl-5-methoxykynurenamine	61	0.1724	0.4513	29%	1.29
9137	Metabolite - 2141	61	0.1725	0.4513	23%	1.23
18232	Metabolite - 5403	50	0.1746	0.4537	16%	1.16
19372	Metabolite - 6269	50	0.1776	0.4565	19%	1.19
542	3-hydroxybutanoic acid	50	0.1785	0.4565	50%	1.50
11323	Metabolite - 2711	61	0.1806	0.4565	72%	1.72
606	uridine	61	0.1806	0.4565	93%	1.93
21631	Metabolite - 8403	50	0.1955	0.4908	24%	1.24
15118	Metabolite - 3784	61	0.2010	0.4996	22%	1.22
22572	Metabolite - 9034	50	0.2035	0.4996	28%	1.28
15121	Metabolite - 3786	61	0.2050	0.4996	-53%	0.47
20950	Metabolite - 7846	50	0.2054	0.4996	-19%	0.81
1649	valine	50	0.2076	0.4996	55%	1.55
1284	threonine	50	0.2098	0.4996	61%	1.61
16992	Metabolite - 4603	61	0.2133	0.4996	38%	1.38
7650	Metabolite - 1834	61	0.2138	0.4996	-19%	0.81
14753	Metabolite - 3663	61	0.2148	0.4996	19%	1.19
17627	Metabolite - 4986	50	0.2153	0.4996	41%	1.41
19919	Metabolite - 6832	61	0.2160	0.4996	-34%	0.66
18969	Metabolite - 5920	61	0.2161	0.4996	46%	1.46
22320	Metabolite - 8889	50	0.2165	0.4996	38%	1.38
12907	cGMP	61	0.2214	0.5078	27%	1.27
14759	Metabolite - 3667	61	0.2265	0.5163	10%	1.10
6379	Metabolite - 1329	61	0.2343	0.5308	28%	1.28
15872	malonic acid	61	0.2395	0.5345	46%	1.46
10825	Metabolite - 2546	61	0.2406	0.5345	-7%	0.93
14988	Metabolite - 3756	61	0.2407	0.5345	-10%	0.90
15990	L-alpha-glycerophosphorylcholine	61	0.2436	0.5345	-35%	0.65
12780	Metabolite - 3098	50	0.2443	0.5345	72%	1.72
9002	Metabolite - 2107	61	0.2446	0.5345	-33%	0.67
16044	Metabolite - 4005	50	0.2553	0.5546	58%	1.58
60	leucine	50	0.2579	0.5570	47%	1.47
1827	riboflavine	61	0.2609	0.5573	44%	1.44
22032	Metabolite - 8766	50	0.2610	0.5573	-13%	0.87
16070	Metabolite - 4019	50	0.2653	0.5613	-15%	0.85
1301	lysine	50	0.2661	0.5613	72%	1.72
6253	Metabolite - 1283	61	0.2689	0.5613	58%	1.58
14043	Metabolite - 3428	61	0.2689	0.5613	-26%	0.74
19513	Metabolite - 6552	61	0.2776	0.5731	-31%	0.69
1708	7,8-dihydrofolic acid	61	0.2791	0.5731	-32%	0.68
8649	Metabolite - 2053	61	0.2796	0.5731	-15%	0.85
15753	hippuric acid	61	0.2839	0.5731	29%	1.29
16232	Isobar 17 includes arginine, N-alpha-acetyl-ornithine	61	0.2839	0.5731	21%	1.21

1591	N-acetyl-L-valine	61	0.2857	0.5731	42%	1.42
19374	Metabolite - 6270	50	0.2867	0.5731	-20%	0.80
15122	glycerol	50	0.2870	0.5731	26%	1.26
15412	Metabolite - 3910	61	0.2889	0.5739	-34%	0.66
2342	serotonin	61	0.2920	0.5770	-54%	0.46
13512	Metabolite - 3315	61	0.2938	0.5776	22%	1.22
22590	Metabolite - 9040	61	0.2957	0.5782	-55%	0.45
12789	Metabolite - 3107	50	0.3012	0.5859	39%	1.39
16138	Metabolite - 4080	50	0.3045	0.5893	-18%	0.82
15681	4-Guanidinobutanoic acid	61	0.3070	0.5908	36%	1.36
1125	isoleucine	50	0.3094	0.5908	43%	1.43
14502	Metabolite - 3539	61	0.3109	0.5908	46%	1.46
14406	Metabolite - 3493	61	0.3119	0.5908	-16%	0.84
1898	proline	61	0.3133	0.5908	10%	1.10
2129	oxitryptan	61	0.3172	0.5952	-17%	0.83
15125	(2-Aminoethyl)phosphonate	61	0.3212	0.5961	-23%	0.77
16226	Isobar 28 includes L-threonine, L-allothreonine, L-homoserine,(S)-(-)-4-amino 2-hydroxybutyric acid	61	0.3213	0.5961	-15%	0.85
6104	tryptamine	50	0.3225	0.5961	26%	1.26
12719	Metabolite - 3055 - possible NH3 adduct of hippuric acid	61	0.3251	0.5980	32%	1.32
15113	Metabolite - 3783	61	0.3303	0.6045	-17%	0.83
16821	Metabolite - 4498	50	0.3358	0.6098	11%	1.11
15743	N,N-dimethylarginine	61	0.3377	0.6098	72%	1.72
12782	Metabolite - 3100	50	0.3397	0.6098	57%	1.57
22570	Metabolite - 9033	50	0.3430	0.6098	-24%	0.76
10147	Metabolite - A-2036	61	0.3459	0.6098	-12%	0.88
21046	digalacturonic acid	61	0.3472	0.6098	-25%	0.75
12773	Metabolite - 3093	50	0.3483	0.6098	24%	1.24
18330	5-methyltetrahydrofolic acid	61	0.3499	0.6098	-21%	0.79
20035	Metabolite - 7008	61	0.3521	0.6098	-13%	0.87
22154	bradykinin	61	0.3530	0.6098	-45%	0.55
22133	DL-hexanoyl-carnitine	61	0.3547	0.6098	50%	1.50
5628	Metabolite - 1086	61	0.3567	0.6098	-33%	0.67
10743	Isobar 4 includes Gluconic acid, DL-arabinose, D-ribose, L-xylose, DL-lyxose, D-xylulose,galactonic acid	61	0.3597	0.6098	13%	1.13
16985	Metabolite - 4600	61	0.3606	0.6098	32%	1.32
1476	glucarate	50	0.3609	0.6098	18%	1.18
17860	Metabolite - 5127	61	0.3632	0.6098	28%	1.28
5466	Metabolite - A-1030	61	0.3635	0.6098	23%	1.23
17845	Metabolite - 5108	61	0.3684	0.6098	17%	1.17
20308	Metabolite - 7270	61	0.3690	0.6098	19%	1.19
18761	Metabolite - 5793	61	0.3694	0.6098	57%	1.57
10476	Metabolite - 2316	61	0.3710	0.6098	-15%	0.85
15382	Metabolite - 3898	61	0.3722	0.6098	17%	1.17
18467	cis-5,8,11,14,17-	61	0.3728	0.6098	32%	1.32

	eicosapentaenoic acid					
15529	Metabolite - 3951	61	0.3733	0.6098	14%	1.14
15336	tartaric acid	61	0.3743	0.6098	16%	1.16
21012	Metabolite - 7889	50	0.3786	0.6098	16%	1.16
12533	Metabolite - 2915	50	0.3809	0.6098	-14%	0.86
10700	Metabolite - 2393	61	0.3831	0.6098	26%	1.26
12753	Metabolite - 3074	50	0.3842	0.6098	15%	1.15
10357	Metabolite - A-2055	61	0.3856	0.6098	-24%	0.76
21763	Metabolite - 8507	61	0.3862	0.6098	-10%	0.90
15074	Metabolite - 3774	61	0.3893	0.6098	18%	1.18
10286	Metabolite - 2272	61	0.3909	0.6098	47%	1.47
14480	Metabolite - 3521	61	0.3909	0.6098	-42%	0.58
13872	Metabolite - 3393	61	0.3933	0.6098	52%	1.52
8300	Metabolite - 1988	61	0.3934	0.6098	-13%	0.87
22586	Metabolite - 9039	61	0.3939	0.6098	-48%	0.52
13920	Metabolite - 3404	61	0.3967	0.6107	16%	1.16
12645	Metabolite - 3017	50	0.3978	0.6107	24%	1.24
18369	gamma-glu-leu	61	0.3997	0.6111	11%	1.11
19368	Metabolite - 6267	50	0.4016	0.6115	29%	1.29
19613	Metabolite - 6670	61	0.4043	0.6131	-20%	0.80
12756	Metabolite - 3077	50	0.4130	0.6237	25%	1.25
7644	Metabolite - 1831	61	0.4185	0.6289	18%	1.18
12639	Metabolite - 3012	50	0.4198	0.6289	46%	1.46
6130	Metabolite - 1208	61	0.4239	0.6324	-41%	0.59
11053	Metabolite - 2567	61	0.4256	0.6324	10%	1.10
17390	Metabolite - 4806	50	0.4286	0.6335	-7%	0.93
14247	Metabolite - 3475	61	0.4319	0.6335	14%	1.14
20234	Metabolite - 7170	61	0.4326	0.6335	25%	1.25
17327	Metabolite - 4767	50	0.4364	0.6335	13%	1.13
17359	Metabolite - 4791	50	0.4383	0.6335	-21%	0.79
22163	EDTA	50	0.4387	0.6335	47%	1.47
8176	Metabolite - 1974	61	0.4407	0.6335	-16%	0.84
1647	glutamine	50	0.4446	0.6335	44%	1.44
17614	Metabolite - 4966	50	0.4467	0.6335	-29%	0.71
5430	Metabolite - A-1008	61	0.4497	0.6335	-5%	0.95
19462	Metabolite - 6446	50	0.4509	0.6335	-9%	0.91
6398	Metabolite - 1335	61	0.4510	0.6335	-19%	0.81
15639	Metabolite - 3984	61	0.4520	0.6335	54%	1.54
12777	Metabolite - 3097	50	0.4558	0.6335	25%	1.25
15805	maltose	50	0.4590	0.6335	25%	1.25
1366	trans-4-hydroxyproline	50	0.4603	0.6335	31%	1.31
4966	xylitol	50	0.4612	0.6335	10%	1.10
1419	5'-s-methyl-5'-thioadenosine	61	0.4617	0.6335	18%	1.18
11777	glycine	50	0.4623	0.6335	36%	1.36
22020	Metabolite - 8749	50	0.4627	0.6335	9%	1.09
21047	3-methyl-2-oxobutyric	61	0.4640	0.6335	-12%	0.88
16290	Metabolite - 4133	50	0.4655	0.6335	-7%	0.93
12803	Metabolite - A-2441	50	0.4671	0.6335	-12%	0.88
8527	Metabolite - A-1931	61	0.4700	0.6335	-3%	0.97

19013	Metabolite - 5931	61	0.4701	0.6335	7%	1.07
22568	Metabolite - 9031	61	0.4758	0.6335	-43%	0.57
12783	Metabolite - 3101	50	0.4760	0.6335	27%	1.27
5533	Metabolite - A-1096	61	0.4762	0.6335	29%	1.29
16805	Metabolite - 4488	61	0.4772	0.6335	21%	1.21
22132	DL-alpha-hydroxyisocaproic acid	61	0.4774	0.6335	15%	1.15
16071	Metabolite - 4020	50	0.4818	0.6360	7%	1.07
22439	Metabolite - 8949	61	0.4839	0.6360	17%	1.17
5765	Metabolite - 1142 - possible 5-hydroxypentanoate or beta-hydroxyisovaleric acid	61	0.4877	0.6360	-13%	0.87
1481	inositol 1-phosphate	50	0.4879	0.6360	-13%	0.87
13217	Metabolite - 3184	61	0.4913	0.6360	15%	1.15
5689	Metabolite - 1111 - possible methylnitronitrosoguanidine or ethyl thiocarbamoylacetate	61	0.4925	0.6360	-19%	0.81
22166	glu-glu	61	0.4985	0.6360	29%	1.29
21127	monopalmitin	50	0.4987	0.6360	-10%	0.90
22601	Metabolite - 9044	50	0.4990	0.6360	25%	1.25
18384	Metabolite - 5487	50	0.5030	0.6360	-12%	0.88
18943	Metabolite - 5912	61	0.5031	0.6360	-13%	0.87
22145	acetyl-L-carnitine	61	0.5078	0.6360	20%	1.20
17486	Metabolite - 4886	61	0.5102	0.6360	-14%	0.86
14639	Metabolite - 3603	61	0.5123	0.6360	16%	1.16
12785	Metabolite - 3103	50	0.5144	0.6360	38%	1.38
9016	Metabolite - 2109	61	0.5147	0.6360	-6%	0.94
10136	Metabolite - A-2034	61	0.5160	0.6360	4%	1.04
1417	kynurenic acid	61	0.5187	0.6360	12%	1.12
16893	Metabolite - 4530	61	0.5188	0.6360	107%	2.07
1564	citric acid	50	0.5189	0.6360	12%	1.12
22001	3-hydroxyoctanoic acid	61	0.5214	0.6360	19%	1.19
18349	DL-indole-3-lactic acid	61	0.5214	0.6360	13%	1.13
16080	Metabolite - 4026	61	0.5228	0.6360	-26%	0.74
15197	1-methylnicotinamide-1	61	0.5243	0.6360	24%	1.24
18172	Metabolite - 5391	61	0.5247	0.6360	-13%	0.87
2240	homogentisate	61	0.5248	0.6360	-6%	0.94
15063	Metabolite - 3772	61	0.5263	0.6360	17%	1.17
5618	Metabolite - 1085 - possible isolobinine or 4-aminoestra-1,3,5(10)-triene-3,17beta-diol	61	0.5287	0.6360	-8%	0.92
59	histidine	61	0.5290	0.6360	-8%	0.92
5478	Metabolite - A-1036	61	0.5339	0.6394	-7%	0.93
21013	Metabolite - 7890	50	0.5363	0.6394	-15%	0.85
22262	Isobar 61 includes 4-methyl-2-oxovaleric acid, 3-methyl-2-oxovaleric acid	61	0.5381	0.6394	-10%	0.90
10850	Metabolite - 2548 - possible Cl adduct of uric acid	61	0.5398	0.6394	21%	1.21
22360	Metabolite - 8901	61	0.5414	0.6394	16%	1.16

5475	Metabolite - A-1033	61	0.5423	0.6394	12%	1.12
8509	Metabolite - 2041	61	0.5445	0.6394	7%	1.07
20694	oxalic acid	61	0.5456	0.6394	-7%	0.93
18010	Metabolite - 5231	61	0.5487	0.6411	33%	1.33
2183	thymidine	61	0.5516	0.6423	10%	1.10
2150	pyridoxamine	61	0.5544	0.6432	-4%	0.96
19708	Metabolite - 6711	61	0.5558	0.6432	-9%	0.91
22130	DL-3-phenyllactic acid	61	0.5598	0.6457	-16%	0.84
16711	Metabolite - 4431	61	0.5614	0.6457	-15%	0.85
20391	Metabolite - 7334	61	0.5785	0.6615	-13%	0.87
16189	Metabolite - 4097	61	0.5787	0.6615	9%	1.09
7177	Metabolite - 1656	61	0.5817	0.6623	36%	1.36
10655	Metabolite - 2388	61	0.5830	0.6623	11%	1.11
16506	Metabolite - 4271	50	0.5872	0.6651	26%	1.26
18146	Metabolite - 5366	50	0.5890	0.6651	-14%	0.86
19494	Metabolite - 6506	50	0.6032	0.6752	-11%	0.89
18109	isocitrate	61	0.6041	0.6752	20%	1.20
1110	arachidonic acid	50	0.6062	0.6752	8%	1.08
17283	Metabolite - 4750	61	0.6064	0.6752	-15%	0.85
13211	Metabolite - 3182	61	0.6084	0.6752	-13%	0.87
12726	Metabolite - 3058	50	0.6104	0.6752	25%	1.25
15996	aspartate	50	0.6134	0.6752	27%	1.27
16705	Metabolite - 4428	61	0.6135	0.6752	13%	1.13
16865	Metabolite - 4522	50	0.6143	0.6752	6%	1.06
12648	Metabolite - 3020	50	0.6166	0.6755	-6%	0.94
6266	Metabolite - 1286	61	0.6183	0.6755	6%	1.06
1105	Linoleic acid	50	0.6208	0.6755	-6%	0.94
15730	suberic acid	61	0.6219	0.6755	19%	1.19
12650	Metabolite - 3022	50	0.6242	0.6761	24%	1.24
20031	Metabolite - 7007	61	0.6307	0.6811	-10%	0.90
16860	Metabolite - 4517	50	0.6346	0.6815	-9%	0.91
17064	Metabolite - 4624	50	0.6349	0.6815	19%	1.19
19934	inositol	50	0.6365	0.6815	11%	1.11
12663	Metabolite - 3030	50	0.6414	0.6836	29%	1.29
20866	Metabolite - 7786	61	0.6422	0.6836	4%	1.04
22602	Metabolite - 9045	50	0.6447	0.6843	-13%	0.87
12593	Metabolite - 2973	50	0.6526	0.6907	3%	1.03
15500	carnitine	61	0.6619	0.6957	-5%	0.95
7595	Metabolite - 1817	61	0.6633	0.6957	5%	1.05
19364	Metabolite - 6246	50	0.6655	0.6957	13%	1.13
20248	Metabolite - 7177	61	0.6667	0.6957	10%	1.10
14054	Metabolite - 3430 - possible gly-leu, acetyl-lys, ala-val	61	0.6670	0.6957	4%	1.04
11438	phosphate	50	0.6686	0.6957	5%	1.05
19511	Metabolite - 6551	61	0.6777	0.7032	6%	1.06
20192	Metabolite - 7146	61	0.6833	0.7061	-9%	0.91
16949	Metabolite - 4592	61	0.6842	0.7061	-7%	0.93
2137	biliverdin	61	0.6893	0.7093	-18%	0.82
19961	Metabolite - 6913	50	0.6913	0.7093	2%	1.02

7107	Metabolite - A-1664	61	0.6945	0.7093	-22%	0.78
18756	Metabolite - 5791	61	0.6950	0.7093	-8%	0.92
12768	Metabolite - 3088	50	0.7007	0.7125	14%	1.14
19362	Metabolite - 6226	50	0.7030	0.7125	-10%	0.90
12791	Metabolite - 3109	50	0.7038	0.7125	18%	1.18
528	alpha-keto-glutarate	61	0.7155	0.7217	17%	1.17
10746	Isobar 6 includes valine, betaine	61	0.7178	0.7217	10%	1.10
10304	Metabolite - 2276	61	0.7191	0.7217	24%	1.24
19377	Metabolite - 6272	50	0.7207	0.7217	9%	1.09
18929	Metabolite - 5907	50	0.7249	0.7239	-9%	0.91
9060	Metabolite - A-1994	61	0.7284	0.7248	16%	1.16
22577	Metabolite - 9035	50	0.7323	0.7248	17%	1.17
1121	heptadecanoic acid	50	0.7328	0.7248	4%	1.04
14840	Metabolite - 3708	61	0.7359	0.7248	5%	1.05
16712	Metabolite - 4432	61	0.7373	0.7248	-3%	0.97
16285	Metabolite - A-2798	50	0.7393	0.7248	-11%	0.89
1584	methyl indole-3-acetate	61	0.7401	0.7248	-13%	0.87
18868	Metabolite - 5847	50	0.7421	0.7248	13%	1.13
16843	Metabolite - 4510	50	0.7452	0.7248	15%	1.15
19800	Metabolite - 6750	61	0.7544	0.7248	24%	1.24
12457	Metabolite - 2892	61	0.7565	0.7248	-9%	0.91
15677	3-methyl-L-histidine	61	0.7568	0.7248	-4%	0.96
57	glutamic acid	50	0.7577	0.7248	18%	1.18
21762	Metabolite - 8506	61	0.7591	0.7248	7%	1.07
12770	Metabolite - 3090	50	0.7605	0.7248	7%	1.07
19367	Metabolite - 6266	50	0.7620	0.7248	-6%	0.94
11770	Metabolite - 2806	61	0.7646	0.7248	5%	1.05
10148	Metabolite - 2257	61	0.7649	0.7248	13%	1.13
10570	Metabolite - 2366	61	0.7663	0.7248	-6%	0.94
20267	Metabolite - 7187	61	0.7675	0.7248	14%	1.14
577	fructose	50	0.7684	0.7248	-7%	0.93
16308	Metabolite - 4147	50	0.7687	0.7248	7%	1.07
6362	Metabolite - 1323 - possible p-cresol sulfate	61	0.7707	0.7249	22%	1.22
12112	Metabolite - A-2314	61	0.7740	0.7261	6%	1.06
12647	Metabolite - 3019	50	0.7767	0.7268	18%	1.18
13104	Metabolite - 3160	61	0.7839	0.7295	-5%	0.95
17060	Metabolite - 4622	61	0.7853	0.7295	7%	1.07
1365	tetradecanoic acid	50	0.7859	0.7295	-5%	0.95
1512	picolinic acid	50	0.7875	0.7295	-5%	0.95
12673	Metabolite - 3040	50	0.7915	0.7315	7%	1.07
12876	Metabolite - 3125	61	0.7960	0.7338	-8%	0.92
18892	Metabolite - 5866	61	0.8003	0.7360	-5%	0.95
12035	nonanate	50	0.8032	0.7368	5%	1.05
18678	Metabolite - 5730	61	0.8065	0.7371	-7%	0.93
19955	Metabolite - 6907	50	0.8075	0.7371	7%	1.07
19323	Docosahexaenoic Acid	50	0.8175	0.7428	3%	1.03
12656	Metabolite - 3025	50	0.8225	0.7428	13%	1.13

12751	Metabolite - 3073	50	0.8227	0.7428	-10%	0.90
18706	Metabolite - 5769	61	0.8236	0.7428	-6%	0.94
15064	Metabolite - 3773	61	0.8252	0.7428	10%	1.10
12666	Metabolite - 3033 - possible threonine deriv-	50	0.8278	0.7428	10%	1.10
16829	Metabolite - 4503	50	0.8286	0.7428	-3%	0.97
12771	Metabolite - 3091	50	0.8298	0.7428	7%	1.07
21188	1-stearoyl-rac-glycerol	50	0.8452	0.7498	3%	1.03
16509	Metabolite - 4273	50	0.8458	0.7498	-8%	0.92
10544	Metabolite - 2329	61	0.8460	0.7498	-9%	0.91
15625	Metabolite - 3976	61	0.8476	0.7498	3%	1.03
6461	Metabolite - A-1329	61	0.8499	0.7498	7%	1.07
17207	Metabolite - 4707	61	0.8507	0.7498	4%	1.04
12008	Metabolite - 2847	61	0.8532	0.7498	6%	1.06
17389	Metabolite - 4796	50	0.8537	0.7498	-14%	0.86
597	phosphoenolpyruvate	61	0.8558	0.7499	-4%	0.96
10111	Metabolite - A-2033	61	0.8587	0.7506	-5%	0.95
8796	Metabolite - 2074	61	0.8687	0.7575	5%	1.05
7029	Metabolite - 1597	61	0.8755	0.7601	2%	1.02
15253	Metabolite - 3832 - possible phenol sulfate	61	0.8757	0.7601	6%	1.06
20194	Metabolite - 7147	61	0.8787	0.7607	-2%	0.98
15535	Metabolite - 3955	61	0.8805	0.7607	6%	1.06
15129	D-alanyl-D-alanine	50	0.8826	0.7608	3%	1.03
12658	Metabolite - 3026	50	0.8933	0.7650	8%	1.08
10604	Metabolite - 2370	61	0.8961	0.7650	-4%	0.96
13179	creatine	61	0.8973	0.7650	-4%	0.96
10781	Metabolite - 2469	61	0.8992	0.7650	5%	1.05
1358	octadecanoic acid	50	0.9002	0.7650	1%	1.01
15365	sn-Glycerol 3-phosphate	50	0.9018	0.7650	3%	1.03
9172	Metabolite - A-2000	61	0.9060	0.7650	-1%	0.99
15227	trans-aconitic acid-1	61	0.9063	0.7650	3%	1.03
5702	choline	61	0.9074	0.7650	-4%	0.96
12625	Metabolite - 3002	50	0.9081	0.7650	2%	1.02
19860	Metabolite - 6784	61	0.9194	0.7716	1%	1.01
12781	Metabolite - 3099	50	0.9201	0.7716	4%	1.04
13273	Metabolite - 3224	61	0.9344	0.7759	3%	1.03
21025	iminodiacetic acid	50	0.9345	0.7759	2%	1.02
12912	Metabolite - 3129	61	0.9367	0.7759	-1%	0.99
6131	Metabolite - 1209	61	0.9387	0.7759	-3%	0.97
6380	Metabolite - 1330	61	0.9399	0.7759	-2%	0.98
2125	taurine	61	0.9400	0.7759	-3%	0.97
18118	Metabolite - 5346	50	0.9423	0.7759	-3%	0.97
19291	Metabolite - 6132	61	0.9430	0.7759	-2%	0.98
12667	Metabolite - 3034	50	0.9440	0.7759	1%	1.01
20927	Metabolite - 7815	61	0.9519	0.7796	-1%	0.99
16665	Metabolite - 4364	50	0.9529	0.7796	-2%	0.98
20228	Metabolite - 7169	61	0.9548	0.7796	-2%	0.98
15600	Metabolite - 3964	61	0.9639	0.7799	-1%	0.99

9905	Metabolite - 2231	61	0.9664	0.7799	0%	1.00
16496	Metabolite - 4251	50	0.9670	0.7799	1%	1.01
513	creatinine	61	0.9687	0.7799	-1%	0.99
22581	Metabolite - 9037	61	0.9712	0.7799	2%	1.02
18882	taurodeoxycholic acid	61	0.9715	0.7799	2%	1.02
12754	Metabolite - 3075	50	0.9801	0.7799	1%	1.01
13018	Metabolite - 3138	61	0.9829	0.7799	1%	1.01
8196	Metabolite - 1979 - Cl adduct of isobar 19	61	0.9833	0.7799	0%	1.00
19397	Metabolite - 6326	50	0.9846	0.7799	-1%	0.99
13288	Metabolite - 3228	61	0.9860	0.7799	0%	1.00
19490	Metabolite - 6488	50	0.9863	0.7799	1%	1.01
12757	Metabolite - 3078	50	0.9867	0.7799	-1%	0.99
12790	Metabolite - 3108	50	0.9880	0.7799	1%	1.01
16512	Metabolite - 4275	50	0.9908	0.7799	0%	1.00
17330	Metabolite - 4769	50	0.9910	0.7799	0%	1.00
19363	Metabolite - 6227	50	0.9929	0.7799	0%	1.00
63	cholesterol	50	0.9929	0.7799	0%	1.00
10782	Metabolite - 2486	61	0.9967	0.7812	0%	1.00
22548	Metabolite - 9026	50	0.9988	0.7812	0%	1.00

[00207] Recursive partitioning statistical analysis of all the metabolites in cardiac tissue between normal and DCM subjects identified Metabolite - 3808 (Metabolite - 3808) as a compound that separated both groups of subjects perfectly (LogWorth = 6.61). Specifically, all subjects with DCM phenotype had levels of Metabolite - 3808 above the cutoff value of 629275 while all the subjects with a normal phenotype had Metabolite - 3808 levels below the cutoff value (Figure 20). The cutoff value in the graph in Figure 20 is indicated by a broken line.

[00208] To evaluate the biomarkers discovered in the mouse model of DCM, analysis of human subjects was performed. Biomarkers were discovered by (1) analyzing plasma samples from different groups of human subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the two groups.

[00209] Two groups of subjects were used. One group consisted of 39 subjects (18 male, 21 female) with dilated cardiomyopathy (DCM). The second group consisted of 31 healthy control subjects (14 male, 17 male). Subjects were balanced for age and gender; mean age of control females was 50.1 +/-10.1 and DCM females was 50.0 +/-11.3 while the mean age of control males was 42.7 +/-11.3 and DCM males was 45.8 +/-10.9.

[00210] T-tests (Table 23) were used to determine differences in the mean levels of metabolites between the two populations (i.e., Dilated Cardiomyopathy, DCM vs. Healthy control). Classification analysis was carried out using random forest analyses to uncover the biomarkers that can best differentiate the two groups. The results of the Random forest analysis are shown in Table 24 and the most important biomarkers useful to classify subjects as healthy or DCM are listed in Table 25.

Biomarkers:

[00211] As listed below in Table 23, biomarkers were discovered that were differentially present between plasma samples collected from dilated cardiomyopathy subjects and healthy subjects.

[00212] Table 23 includes, for each listed biomarker, the p-value and q-value determined in the statistical analysis of the data concerning the biomarkers and an indication of the percentage difference in the dilated cardiomyopathy mean level as compared to the healthy mean level in plasma. "ID" refers to the compound identification number used as a primary key for that compound in the in-house chemical database. "Library" indicates the chemical library that was used to identify the compounds. The number 50 refers to the GC library and the numbers 200 and 201 refer to the LC library. "Mouse" indicates the compounds that were also biomarkers discovered in the mouse model of DCM.

Table 23. Biomarkers for DCM

ID	Library	Compound	*p*-value	*q*-value	*%Change in DCM*	*Mouse*
32873	201	Metabolite - 11556	5.943E-07	0.0001	-10%	
1558	200	4-acetamidobutanoate	6.339E-06	0.0005	7%	
32709	200	Metabolite - 03056	1.925E-05	0.001	73%	
33510	200	Metabolite - 12095	2.318E-05	0.001	-9%	
33442	200	pseudouridine	3.734E-05	0.0012	1%	
32425	201	dehydroisoandrosterone sulfate (DHEA-S)	0.0001	0.0012	4%	
32519	200	Metabolite - 11205	0.0001	0.0012	14%	
32637	201	Metabolite - 11320	0.0001	0.0012	6%	
16866	50	Metabolite - 04523	0.0001	0.0015	9%	
1114	201	deoxycholate	0.0001	0.0017	11%	
1284	200	threonine	0.0001	0.0017	1%	Yes

32675	200	Metabolite - 03951	0.0001	0.0017	12%	
15506	200	choline	0.0002	0.002	29%	Yes
32652	200	Metabolite - 11335	0.0002	0.0029	-31%	
32197	201	3-(4-hydroxyphenyl)lactate	0.0003	0.0035	-4%	Yes
33515	200	Metabolite - 12100	0.0003	0.0036	31%	
18929	50	Metabolite - 05907	0.0004	0.004	-3%	
32405	200	3-indolepropionate	0.0004	0.004	-13%	
19934	50	myo-inositol	0.0006	0.0051	17%	Yes
33453	50	alpha-ketoglutarate	0.0006	0.0055	2%	
1712	200	cortisol	0.0007	0.006	-53%	
32807	201	Metabolite - 11490	0.0012	0.0094	-12%	
25607	50	Metabolite - 10437	0.0013	0.0097	4%	
25459	50	Metabolite - 10395	0.0015	0.0104	-3%	
33477	50	erythronate*	0.0016	0.0106	-25%	
32635	201	Metabolite - 11318	0.0018	0.0117	17%	
57	50	glutamate	0.002	0.0126	-5%	Yes
18254	200	paraxanthine	0.0021	0.0126	-3%	
33973	201	epiandrosterone sulfate	0.0021	0.0126	51%	
32560	201	Metabolite - 07765	0.0025	0.0142	-4%	
20699	50	erythritol	0.0026	0.0144	-19%	Yes
32590	201	Metabolite - 11273	0.003	0.0154	-18%	
32619	201	Metabolite - 11302	0.003	0.0154	-11%	
22602	50	Metabolite - 09045	0.0033	0.0161	-6%	
32829	200	Metabolite - 03653	0.0033	0.0161	-4%	
32910	201	Metabolite - 11593	0.0035	0.0168	-8%	
32599	201	Metabolite - 11282	0.0037	0.017	3%	
16653	50	Metabolite - 04361	0.004	0.0176	2%	
18232	50	Metabolite - 05403	0.004	0.0176	30%	
15677	201	3-methylhistidine	0.0048	0.0206	120%	Yes
12770	50	Metabolite - 03090	0.0055	0.0225	13%	
31591	201	androsterone sulfate	0.0055	0.0225	-78%	
584	50	mannose	0.0063	0.0248	16%	Yes
15140	200	kynurenine	0.0067	0.0258	22%	Yes
33206	201	Metabolite - 11861	0.0068	0.0258	-17%	
33144	200	Metabolite - 11799	0.007	0.026	-13%	
33507	200	Metabolite - 12092	0.0076	0.0278	-3%	
32740	201	Metabolite - 11423	0.0078	0.0278	-27%	
33652	201	Metabolite - 12230	0.008	0.0279	-3%	
53	200	glutamine	0.0082	0.028	2%	Yes
32808	201	Metabolite - 11491	0.0087	0.0289	39%	
33516	200	Metabolite - 12101	0.0089	0.0289	80%	
3147	50	xanthine	0.009	0.0289	20%	
12753	50	Metabolite - 03074	0.0099	0.0309	-5%	
1303	50	malate	0.0101	0.0309	3%	Yes
32762	201	Metabolite - 11445	0.0101	0.0309	4%	
2734	200	gamma-glutamyltyrosine	0.0115	0.0339	-4%	Yes
32718	200	Metabolite - 01342	0.0116	0.0339	26%	
32644	200	Metabolite - 11327	0.0117	0.0339	-25%	
32654	200	Metabolite - 11337	0.012	0.0341	9%	

33937	201	alpha-hydroxyisovalerate	0.0127	0.0355	38%	
12017	200	3-methoxytyrosine	0.0134	0.0369	7%	
32753	201	Metabolite - 09789	0.0154	0.0418	-9%	
32587	201	Metabolite - 02249	0.016	0.0427	44%	
59	201	histidine	0.0179	0.047	-1%	Yes
22548	50	Metabolite - 09026	0.0188	0.0486	28%	
16959	50	Metabolite - 04595	0.0191	0.0488	21%	
33422	200	gammaglutamylphenylalanine	0.0197	0.0494	30%	
64	200	phenylalanine	0.0211	0.0522	-4%	Yes
32110	50	Metabolite - 11086	0.0227	0.055	8%	
33132	200	Metabolite - 11787	0.0229	0.055	132%	
32859	200	Metabolite - 11542	0.0236	0.0557	-19%	
32672	200	Metabolite - 02546	0.024	0.0557	-5%	
21044	50	2-hydroxybutyrate (AHB)	0.0244	0.0557	3%	Yes
1356	201	nonadecanoate (19:0)	0.025	0.0557	-1%	
32198	200	acetylcarnitine	0.025	0.0557	-44%	Yes
32830	200	Metabolite - 11513	0.0251	0.0557	1%	
599	50	pyruvate	0.0261	0.0571	-7%	
1358	201	stearate (18:0)	0.0269	0.0576	-13%	Yes
32701	200	urate*	0.027	0.0576	-2%	Yes
32393	200	glutamylvaline	0.0276	0.0576	11%	
24077	50	Metabolite - 09727	0.0277	0.0576	-12%	
24076	50	Metabolite - 09726	0.0285	0.0586	-31%	
1299	200	tyrosine	0.0288	0.0587	-3%	Yes
27718	200	creatine	0.0294	0.059	-29%	Yes
12757	50	Metabolite - 03078	0.0299	0.0594	19%	
1769	200	cortisone	0.0308	0.0604	33%	
32836	200	HWESASXX*	0.0315	0.0612	11%	
33028	200	Metabolite - 01497	0.0325	0.0624	-8%	
33961	200	1-stearoylglycerophosphocholine	0.0329	0.0624	4%	
33157	200	Metabolite - 11812	0.0334	0.0624	27%	
32606	201	bilirubin*	0.0336	0.0624	2%	
33939	201	N-acetylthreonine	0.0358	0.0659	7%	
25609	50	Metabolite - 10439	0.0363	0.066	75%	
19368	50	Metabolite - 06267	0.0367	0.066	-7%	
12789	50	Metabolite - 03107	0.0384	0.0683	17%	Yes
33441	200	isobutyrylcarnitine	0.0391	0.0684	-13%	
34035	201	linolenate [alpha or gamma; (18:3(n-3 or 6))]	0.0394	0.0684	-2%	
33363	200	gamma-glutamylmethionine*	0.0396	0.0684	-17%	
30257	50	Metabolite - 10729	0.0417	0.0712	3%	
33821	200	Metabolite - 12393	0.0431	0.0724	0%	
33389	201	Metabolite - 12038	0.0432	0.0724	3%	
21047	201	3-methyl-2-oxobutyrate	0.0464	0.077	15%	Yes
18349	200	indolelactate	0.0472	0.0775	-55%	Yes
12110	200	isocitrate	0.0478	0.0775	11%	
33405	200	Metabolite - 12053	0.0481	0.0775	0%	
32497	201	10c-undecenoate	0.0496	0.0792	17%	
33738	201	Metabolite - 12316	0.0502	0.0794	-14%	

19402	50	Metabolite - 06346	0.0518	0.0812	53%	
33964	200	[H]HWESASLLR[OH]	0.0523	0.0813	26%	
12795	50	Metabolite - 03113	0.0535	0.0824	6%	
32754	201	Metabolite - 11437	0.0542	0.0827	-13%	
18497	201	taurocholate	0.055	0.0831	-9%	
1508	200	pantothenate	0.0565	0.0847	25%	Yes
32625	201	Metabolite - 11308	0.0609	0.0904	-50%	
32729	200	Metabolite - 11412	0.0621	0.0915	-26%	
31555	201	pyridoxate	0.0648	0.0946	18%	
33960	200	1-oleoylglycerophosphocholine	0.0664	0.0961	9%	
1642	201	caprate (10:0)	0.068	0.0976	-5%	
31454	50	cystine	0.0697	0.0985	-11%	
18477	200	glycodeoxycholate	0.07	0.0985	-44%	
32850	201	Metabolite - 11533	0.0711	0.0985	155%	
22895	50	Metabolite - 09299	0.0717	0.0985	-15%	
31618	50	Metabolite - 10964	0.0718	0.0985	-25%	
34007	50	Metabolite - 12502	0.072	0.0985	-12%	
1638	200	arginine	0.0748	0.1014	-14%	Yes
33852	200	Metabolite - 12424	0.0767	0.1031	-20%	
33420	50	gamma-tocopherol*	0.0798	0.1058	-17%	
32398	200	sebacate	0.0799	0.1058	-9%	
33403	200	Metabolite - 12051	0.082	0.1077	-10%	
33957	200	1-heptadecanoylglycerophosphocholine	0.0838	0.1085	-1%	
32518	200	Metabolite - 11204	0.0849	0.1091	27%	
1361	201	pentadecanoate (15:0)	0.0887	0.1131	69%	
1645	201	laurate (12:0)	0.0906	0.1141	10%	
32620	201	Metabolite - 11303	0.0908	0.1141	3%	
15990	200	glycerophosphorylcholine (GPC)	0.0928	0.1158	-9%	Yes
27531	201	hyodeoxycholate	0.0956	0.1184	2%	
1105	201	linoleate (18:2(n-6))	0.0969	0.1184	66%	Yes
33140	200	Metabolite - 11795	0.097	0.1184	-1%	
16308	50	Metabolite - 04147	0.099	0.1193	38%	
33927	200	Metabolite - 12481	0.0994	0.1193	18%	
19363	50	Metabolite - 06227	0.0998	0.1193	16%	
31509	50	Metabolite - 10931	0.1008	0.1196	31%	
32561	201	Metabolite - 11244	0.1052	0.1239	-11%	
32846	201	Metabolite - 11529	0.1091	0.1257	253%	
21630	50	Metabolite - 08402	0.1098	0.1257	2%	
32550	201	Metabolite - 02272	0.1102	0.1257	21%	
1107	50	allantoin	0.1104	0.1257	-13%	Yes
32867	201	Metabolite - 11550	0.1104	0.1257	5%	
32549	201	Metabolite - 02269	0.1157	0.1309	8%	
32786	200	Metabolite - 11469	0.1181	0.1321	8%	
32501	201	dihomo-alpha-linolenate (20:3(n-3))	0.1188	0.1321	24%	
21128	50	octadecanol	0.1191	0.1321	11%	
16819	50	Metabolite - 04496	0.12	0.1323	-7%	
33209	201	Metabolite - 11864	0.121	0.1324	-21%	
32778	200	Metabolite - 11461	0.1216	0.1324	-6%	
32839	201	Metabolite - 11522	0.1284	0.1389	20%	

32868	201	glycocholate*	0.1304	0.1401	-28%	
33969	201	stearidonate (18:4(n-3))	0.1388	0.146	89%	
12783	50	Metabolite - 03101	0.1405	0.146	21%	
31453	50	cysteine	0.141	0.146	16%	
33103	50	Metabolite - 11758	0.1417	0.146	26%	
32758	201	Metabolite - 11441	0.1421	0.146	24%	
33935	200	piperine	0.1422	0.146	-26%	
33472	200	Metabolite - 12085	0.1426	0.146	3%	
32978	200	Metabolite - 11656	0.1427	0.146	-26%	
32504	201	n-3 DPA (22:5(n-3))	0.1448	0.1463	-3%	
32877	201	Metabolite - 11560	0.1463	0.1463	47%	
27273	50	Metabolite - 10506	0.1465	0.1463	36%	
19370	50	Metabolite - 06268	0.149	0.1472	2%	
1572	50	glycerate	0.1506	0.1472	1%	Yes
32346	201	glycochenodeoxycholate	0.1506	0.1472	13%	
32769	201	Metabolite - 11452	0.1513	0.1472	-17%	
11777	50	glycine	0.1564	0.1508	-3%	Yes
32759	201	Metabolite - 11442	0.1572	0.1508	79%	
513	200	creatinine	0.161	0.1537	-2%	Yes
32452	200	propionylcarnitine	0.163	0.1547	9%	
20675	201	1,5-anhydroglucitol (1,5-AG)	0.1674	0.1573	5%	
22600	50	Metabolite - 09043	0.1677	0.1573	56%	
33380	201	Metabolite - 12029	0.1692	0.1573	-5%	
25532	50	Metabolite - 10413	0.1695	0.1573	-7%	
15335	50	mannitol	0.173	0.1598	2%	
32952	201	Metabolite - 02277	0.1747	0.1605	-13%	
27275	50	Metabolite - 10507	0.1777	0.1617	-8%	
25522	50	Metabolite - 10407	0.178	0.1617	37%	
18335	50	quinate	0.1831	0.1648	17%	
1670	50	urea	0.1833	0.1648	-2%	Yes
31266	50	fructose	0.1853	0.1652	11%	
32401	200	trigonelline (N'-methylnicotinate)	0.1856	0.1652	30%	
33228	200	Metabolite - 11883	0.1941	0.1718	-4%	
32776	200	Metabolite - 11459	0.1997	0.1754	-13%	
1121	201	margarate (17:0)	0.2008	0.1754	12%	Yes
33955	200	1-palmitoylglycerophosphocholine	0.2012	0.1754	-25%	
11438	50	phosphate	0.2028	0.1759	-7%	Yes
32756	201	Metabolite - 02276	0.205	0.1769	-23%	
21127	50	1-palmitoylglycerol (1-monopalmitin)	0.212	0.182	-40%	Yes
1359	201	oleate(18:1(n-9))	0.2134	0.1823	-20%	
16665	50	Metabolite - 04364	0.2221	0.1888	63%	
33662	200	Metabolite - 12240	0.2242	0.1896	17%	
32572	200	Metabolite - 11255	0.2263	0.1899	12%	
32814	201	Metabolite - 11497	0.2274	0.1899	16%	
12774	50	Metabolite - 03094	0.2288	0.1899	-11%	
33774	201	Metabolite - 12349	0.2289	0.1899	33%	
33386	50	Metabolite - 12035	0.233	0.1905	10%	
33415	201	Metabolite - 12063	0.2331	0.1905	21%	
33846	200	indoleacetate*	0.2365	0.1909	-16%	

21049	50	1,6-anhydroglucose	0.2368	0.1909	-27%	
16650	50	Metabolite - 04360	0.2369	0.1909	-40%	
15365	50	glycerol 3-phosphate (G3P)	0.2384	0.1913	12%	Yes
22189	200	palmitoylcarnitine	0.2412	0.1926	22%	
30821	50	Metabolite - 10812	0.2425	0.1927	-47%	
33620	200	Metabolite - 12199	0.2445	0.1934	16%	
12129	200	beta-hydroxyisovalerate	0.2499	0.1967	-3%	Yes
33408	200	Metabolite - 12056	0.2568	0.1992	7%	
31373	50	Metabolite - 10878	0.2571	0.1992	-10%	
32792	201	Metabolite - 11475	0.2573	0.1992	-17%	
18392	200	theobromine	0.2577	0.1992	-10%	
19323	201	docosahexaenoate (DHA; 22:6(n-3))	0.2666	0.2052	-14%	Yes
18394	201	theophylline	0.2846	0.218	-6%	
32795	201	Metabolite - 11478	0.2895	0.2208	-10%	
32698	200	Metabolite - 11381	0.2935	0.2219	-26%	
32412	200	butyrylcarnitine	0.2945	0.2219	0%	
32800	201	Metabolite - 11483	0.296	0.2219	0%	
33198	201	Metabolite - 11853	0.2979	0.2219	-50%	
33254	201	Metabolite - 11909	0.2988	0.2219	-26%	
22842	200	cholate	0.2993	0.2219	38%	
33390	201	Metabolite - 12039	0.3001	0.2219	-22%	
12626	50	Metabolite - 03003	0.3038	0.2237	13%	
12261	201	taurodeoxycholic acid	0.3087	0.2264	-46%	
32578	200	Metabolite - 11261	0.3152	0.2301	-18%	
18868	50	Metabolite - 05847	0.3218	0.2328	-1%	
32735	200	Metabolite - 01911	0.3229	0.2328	-12%	
27719	50	galactonic acid	0.3253	0.233	-14%	
15122	50	glycerol	0.3269	0.233	16%	Yes
33204	201	Metabolite - 11859	0.3274	0.233	3%	
32328	200	hexanoylcarnitine	0.3294	0.2334	-17%	
1898	200	proline	0.3309	0.2334	-41%	Yes
21421	50	Metabolite - 08214	0.3322	0.2334	2%	
32813	201	Metabolite - 11496	0.335	0.2334	-4%	
32697	200	Metabolite - 11380	0.3352	0.2334	74%	
22320	50	Metabolite - 08889	0.3383	0.2334	48%	
32634	201	Metabolite - 11317	0.3387	0.2334	41%	
33194	201	Metabolite - 11849	0.3388	0.2334	-34%	
527	50	lactate	0.3402	0.2335	95%	Yes
33154	200	Metabolite - 11809	0.3458	0.2356	-18%	
32492	201	caprylate (8:0)	0.3467	0.2356	4%	
32838	200	Metabolite - 11521	0.3511	0.2371	-6%	
32616	201	Metabolite - 11299	0.3571	0.2402	-26%	
22154	200	bradykinin	0.3614	0.2422	-4%	Yes
32875	200	Metabolite - 11558	0.3753	0.2495	-33%	
32971	200	Metabolite - 11654	0.3753	0.2495	-11%	
16634	50	Metabolite - 04357	0.3868	0.2551	2%	
19576	50	Metabolite - 06627	0.3898	0.2562	-1%	
33570	200	Metabolite - 12154	0.3919	0.2565	99%	
2137	200	biliverdin	0.3952	0.2577	27%	Yes

32854	200	Metabolite - 11537	0.4062	0.2638	-3%	
17747	200	sphingosine	0.4125	0.2669	-20%	
1365	201	myristate (14:0)	0.4169	0.2683	66%	Yes
32511	201	EDTA*	0.4192	0.2683	63%	
32767	201	Metabolite - 11450	0.4192	0.2683	7%	
32847	201	Metabolite - 11530	0.4303	0.2743	10%	
17805	201	dihomolinolenate (20:2(n-6))	0.4331	0.2743	15%	
32793	200	Metabolite - 11476	0.4346	0.2743	88%	
12781	50	Metabolite - 03099	0.4351	0.2743	64%	
1648	50	serine	0.4384	0.2753	-10%	Yes
32557	201	Metabolite - 06126	0.4398	0.2753	-3%	
1301	50	lysine	0.4438	0.2767	24%	Yes
1126	50	alanine	0.4497	0.2786	2%	Yes
569	200	caffeine	0.4501	0.2786	25%	
32732	201	Metabolite - 11415	0.4532	0.2788	-2%	
33089	50	Metabolite - 11744	0.4537	0.2788	64%	
21184	200	oleoylglycerol (monoolein)	0.4564	0.2795	150%	
22481	50	Metabolite - 08988	0.4648	0.2828	-24%	
18369	200	gamma-glutamylleucine	0.4651	0.2828	-9%	Yes
20299	50	Metabolite - 07266	0.4717	0.2858	14%	
33882	201	Metabolite - 12440	0.4802	0.2895	13%	
1336	201	palmitate (16:0)	0.4822	0.2895	6%	Yes
20489	50	glucose	0.4829	0.2895	46%	
12764	50	Metabolite - 03084	0.4883	0.2907	-66%	
1493	200	ornithine	0.4906	0.2911	-6%	Yes
32595	200	Metabolite - 08893	0.4954	0.2929	40%	
33968	201	5-dodecenoate (12:1(n-7))	0.4994	0.2942	-1%	
12761	50	Metabolite - 03081	0.5076	0.298	20%	
19374	50	Metabolite - 06270	0.5136	0.2988	14%	
22116	201	4-methyl-2-oxopentanoate	0.5162	0.2988	-52%	
33447	201	palmitoleate (16:1(n-7))	0.5171	0.2988	-12%	
32656	201	Metabolite - 11339	0.5186	0.2988	-3%	
32669	200	Metabolite - 11352	0.5188	0.2988	-11%	
542	200	3-hydroxybutyrate (BHBA)	0.5194	0.2988	1%	Yes
31401	50	Metabolite - 10892	0.5325	0.3049	16%	
32319	50	trans-4-hydroxyproline	0.5336	0.3049	137%	
1302	200	methionine	0.5401	0.3068	142%	Yes
32855	201	Metabolite - 11538	0.543	0.3068	10%	
12785	50	Metabolite - 03103	0.5441	0.3068	18%	
32553	201	Metabolite - 03832	0.5469	0.3073	0%	
32869	200	Metabolite - 11552	0.5508	0.308	103%	
12782	50	Metabolite - 03100	0.553	0.308	38%	
2730	200	gamma-glutamylglutamine	0.5534	0.308	71%	
1564	50	citrate	0.5579	0.3089	18%	Yes
32761	201	Metabolite - 11444	0.5587	0.3089	15%	
32632	200	Metabolite - 11315	0.561	0.3092	37%	
1605	201	ursodeoxycholate	0.5703	0.3124	2%	
12593	50	Metabolite - 02973	0.5707	0.3124	44%	
32885	200	Metabolite - 11568	0.5732	0.3124	-4%	

32564	201	Metabolite - 11247	0.5743	0.3124	-9%	
3127	200	hypoxanthine	0.5759	0.3124	28%	
1444	200	pipecolate	0.5811	0.3138	-7%	
1644	201	heptanoate	0.5823	0.3138	-11%	
33227	201	Metabolite - 11882	0.5902	0.3171	13%	
54	200	tryptophan	0.598	0.3203	-7%	Yes
32418	201	myristoleate (14:1(n-5))	0.6043	0.3215	95%	
15753	201	hippurate	0.6049	0.3215	0%	Yes
32774	200	Metabolite - 11457	0.6122	0.3215	-49%	
32648	201	Metabolite - 11331	0.6136	0.3215	-4%	
27710	50	N-acetylglycine	0.615	0.3215	-19%	
606	201	uridine	0.6154	0.3215	-4%	Yes
32797	201	Metabolite - 11480	0.6159	0.3215	35%	
31787	201	3-carboxy - 4-methyl-5-propyl-2-furanpropanoate (CMPF)	0.6206	0.3215	-60%	
32586	200	Metabolite - 01327	0.6213	0.3215	-1%	
32348	200	2-aminobutyrate	0.6217	0.3215	17%	
31489	50	Metabolite - 10914	0.6229	0.3215	8%	
32748	201	Metabolite - 11431	0.6286	0.3235	-21%	
32815	201	Metabolite - 11498	0.6324	0.3235	-9%	
33138	200	Metabolite - 11793	0.6362	0.3245	32%	
12790	50	Metabolite - 03108	0.6404	0.3249	-8%	
12035	201	pelargonate (9:0)	0.6409	0.3249	28%	Yes
27722	50	erythrose	0.6483	0.3277	-6%	
33901	201	Metabolite - 12456	0.6545	0.3298	-12%	
15500	200	carnitine	0.6744	0.3389	136%	Yes
33195	201	Metabolite - 11850	0.6796	0.3405	-9%	
594	201	nicotinamide	0.6834	0.3406	-26%	
33638	201	Metabolite - 12217	0.6867	0.3406	31%	
32593	200	Metabolite - 02036	0.6873	0.3406	-13%	
16511	50	Metabolite - 04274	0.6879	0.3406	-1%	
17627	50	Metabolite - 04986	0.6934	0.3409	-3%	
12767	50	Metabolite - 03087	0.696	0.3409	-5%	
20694	50	oxalate (ethanedioate)	0.6974	0.3409	-8%	Yes
27672	201	3-indoxyl sulfate	0.6979	0.3409	82%	
15676	201	3-methyl-2-oxovalerate	0.6983	0.3409	-9%	
1561	50	alpha-tocopherol	0.7092	0.3442	-6%	Yes
32458	200	oleamide	0.7112	0.3442	8%	
32342	200	adenosine 5'-monophosphate (AMP)	0.7171	0.3458	-12%	
33131	200	Metabolite - 11786	0.7186	0.3458	4%	
33941	200	decanoylcarnitine	0.7205	0.3458	19%	
27278	50	Metabolite - 10510	0.7254	0.3472	3%	
32970	201	Metabolite - 11653	0.7333	0.35	-12%	
32562	201	Metabolite - 11245	0.736	0.3503	27%	
21631	50	Metabolite - 08403	0.7389	0.3507	-20%	
33230	200	Metabolite - 11885	0.7422	0.3513	-24%	
587	50	gluconate	0.7493	0.3526	-38%	
16508	50	Metabolite - 04272	0.7548	0.3526	-8%	
33587	201	eicosenoate [9 or 11, cis or trans]	0.7575	0.3526	1446%	

24074	50	Metabolite - 09706	0.7579	0.3526	45%	
15737	50	glycolate (hydroxyacetate)	0.7599	0.3526	15%	Yes
32489	201	caproate (6:0)	0.7624	0.3526	-24%	
32636	201	Metabolite - 11319	0.7652	0.3526	24%	
33833	201	Metabolite - 12405	0.7667	0.3526	52%	
32863	201	Metabolite - 11546	0.77	0.3526	55%	
27738	50	threonate	0.7742	0.3526	23%	
63	50	cholesterol	0.7753	0.3526	104%	Yes
33402	200	Metabolite - 12050	0.7784	0.3526	40%	
32651	200	Metabolite - 11334	0.7794	0.3526	37%	
33265	200	Metabolite - 11920	0.7797	0.3526	1%	
32757	201	Metabolite - 11440	0.78	0.3526	71%	
512	50	asparagine	0.7878	0.3551	65%	Yes
32857	200	Metabolite - 11540	0.7901	0.3552	54%	
31617	50	Metabolite - 10963	0.7929	0.3555	61%	
32738	200	Metabolite - 11421	0.8008	0.3571	-28%	
27256	50	Metabolite - 10500	0.8059	0.3571	-4%	
32558	201	p-cresol sulfate*	0.8079	0.3571	2%	
27447	201	linoleoylglycerol (monolinolein)	0.808	0.3571	20%	
1125	200	isoleucine	0.8081	0.3571	-11%	Yes
16837	50	Metabolite - 04507	0.8101	0.3571	-42%	
60	200	leucine	0.8141	0.3572	-5%	Yes
1494	200	5-oxoproline	0.8154	0.3572	31%	Yes
33520	200	Metabolite - 12105	0.819	0.3579	-2%	
1110	201	arachidonate (20:4(n-6))	0.8245	0.3594	-14%	Yes
33972	201	10-nonadecenoate (19:1(n-9))	0.8354	0.3613	17%	
16666	50	Metabolite - 04365	0.8692	0.3741	5%	
33971	201	10-heptadecenoate (17:1(n-7))	0.8763	0.3761	-16%	
27264	50	Metabolite - 10503	0.8865	0.3786	-14%	
22570	50	Metabolite - 09033	0.8891	0.3788	2%	
12771	50	Metabolite - 03091	0.8928	0.3794	74%	
19490	50	Metabolite - 06488	0.8965	0.3801	108%	
32548	201	Metabolite - 11231	0.9079	0.383	2%	
21188	50	stearoylglycerol (monostearin)	0.909	0.383	49%	Yes
33488	50	lathosterol	0.9118	0.383	24%	
15630	200	N-acetylornithine	0.9125	0.383	-5%	
21011	50	Metabolite - 07888	0.9175	0.3837	-5%	
32848	201	Metabolite - 11531	0.9202	0.3837	-11%	
33163	200	Metabolite - 11818	0.9209	0.3837	-11%	
15996	50	aspartate	0.9274	0.3855	-15%	Yes
1649	200	valine	0.9428	0.391	61%	Yes
2132	200	citrulline	0.9557	0.3939	31%	Yes
25602	50	Metabolite - 10432	0.9562	0.3939	-21%	
32760	201	Metabolite - 11443	0.9578	0.3939	9%	
33936	200	octanoylcarnitine	0.9591	0.3939	-2%	
33369	50	Metabolite - 12023	0.9705	0.3976	-35%	
3141	200	betaine	0.9845	0.4022	-18%	
32520	200	Metabolite - 11206	0.9863	0.4022	-21%	

[00213] Pathway trend analysis showed strong differentiation of DCM patients in energy and lipid pathways, suggesting TCA cycle inhibition, glucogenic amino acid mobilization, and β-oxidation increases. Adrenergic steroids (cortisol, cortisone) were increased, consistent with general stress, and androgen metabolites (DHEA-S) were strongly diminished in DCM patients, resulting in an apparent metabolic "feminization" of DCM males.

[00214] Comparison to the previous transgenic mouse DCM model plasma study showed that eight compounds, including urate, malate, tyrosine, phenylalanine, erythritol, and others exhibited similar responses and were strongly significant in both studies. Another 16 that were strongly significant in the human study trended in a similar manner in the mouse study. These included α-ketoglutarate, isocitrate, pantothenate, *myo*-inositol, and glutamate. The data confirm that metabolomic profiles of plasma reflect the disease state in human DCM patients, and that the transgenic mouse model shares many of the biomarker alterations associated with human disease.

[00215] T-tests are used to determine if the population means are different, but do not tell us about individual observations. Random Forest analysis is a multivariate technique for identifying compounds that distinguish the Groups. Random forests are used to classify individuals. Random forests are based on a consensus of a large number of decision trees; it is an extremely effective multivariate technique, being resistant to outliers, insensitive to method of normalization, and possesses highly predictive ability for new samples. Shown in Table 24 are results of using the biomarkers listed in Table 23 to classify the subjects as "Healthy" or "DCM". The subjects are correctly classified as Healthy (Control) 81% of the time and correctly classified as having DCM 72% of the time. Subjects are correctly classified with >75% accuracy overall.

Table 24 Random Forest Classification of DCM and Healthy subjects

	Control	DCM	error
Control	25	6	19%
DCM	11	28	28%
OOB estimate of error rate: 24.29%			

[00216] The biomarkers that are most important to correctly classify subjects are shown in Table 25 and the Importance plot is shown in Figure #.

Table 25. Important DCM biomarkers

Metabolite - 11556
4-acetamidobutanoate
Metabolite - 03951
Choline
Metabolite - 03056
Metabolite - 11335
Metabolite - 4523
erythronate
Metabolite - 11593
pseudouridine
Metabolite - 10395
Metabolite - 12095
myo-inositol
3-indolepropionate
deoxycholate
Metabolite - 11320
Metabolite - 3090
Metabolite - 5907
3-(4-hydroxyphenyl)lactate
Metabolite - 11490
paraxanthine
Metabolite - 11542
cortisol
Metabolite - 4361
creatine
Metabolite - 03653
Metabolite - 11282
kynurenine

3D: Biomarkers of obesity; Metabolites that are differentially present in lean compared to obese subjects

[00217] Biomarkers were discovered by (1) analyzing blood samples drawn from different groups of human subjects to determine the levels of metabolites in the samples and then (2) statistically analyzing the results to determine those metabolites that were differentially present in the two groups.

[00218] The plasma samples used for the analysis were from 40 lean subjects (BMI<25) and 40 obese subjects (BMI>30) that had been matched for age and gender. After the levels of metabolites were determined, the data was analyzed using univariate T-tests (i.e., Welch's T-test).

[00219] T-tests were used to determine differences in the mean levels of metabolites between the two populations (i.e., Obese vs. Lean).

Biomarkers:

[00220] As listed below in Table 26, biomarkers were discovered that were differentially present between samples from obese subjects and lean subjects.

[00221] Table 26 includes, for each listed biomarker, the p-value and q-value determined in the statistical analysis of the data concerning the biomarkers and an indication of the obese mean level, lean mean level, and the ratio of obese mean level to lean mean level (Table 26). The term "Isobar" as used in the table indicates the compounds that could not be distinguished from each other on the analytical platform used in the analysis (i.e., the compounds in an isobar elute at nearly the same time and have similar (and sometimes exactly the same) quant ions, and thus cannot be distinguished). Comp_ID refers to the compound identification number used as a primary key for that compound in the in-house chemical database. Library indicates the chemical library that was used to identify the compounds. The number 50 refers to the GC library and the number 61 refers to the LC library.

Table 26. Metabolite biomarkers that are differentially present in obese compared to lean subjects.

COMP_ID	Library	COMPOUND	p-value	q-value	Obese/ Lean	Mean LEAN	Mean OBESE
584	50	mannose	<0.0001	<0.0001	1.873	0.71	1.33
20489	50	D-glucose	<0.0001	<0.0001	1.500	0.78	1.17
18369	61	gamma-glu-leu	<0.0001	<0.0001	1.407	0.86	1.21
20675	50	1-5-anhydro-D-glucitol	<0.0001	<0.0001	0.629	1.24	0.78
1494	50	5-oxoproline	<0.0001	<0.0001	0.433	1.57	0.68
15365	50	sn-glycerol-3-phosphate	<0.0001	<0.0001	0.330	1.85	0.61
527	50	lactate	<0.0001	<0.0001	0.263	1.86	0.49
22803	61	Isobar-66-includes-	<0.0001	1.00E-04	0.207	3.92	0.81
3127	61	hypoxanthine	<0.0001	1.00E-04	0.166	2.9	0.48
25402	50	Metabolite - 10360	<0.0001	<0.0001	2.544	0.57	1.45
5652	61	Metabolite - 1090	<0.0001	<0.0001	0.200	1.7	0.34
7650	61	Metabolite - 1834	<0.0001	<0.0001	0.311	1.8	0.56
8959	61	Metabolite - 2100	<0.0001	<0.0001	0.202	5.09	1.03
10087	61	Metabolite - 2249	<0.0001	<0.0001	1.841	0.82	1.51
11053	61	Metabolite - 2567	<0.0001	<0.0001	1.457	0.81	1.18
12667	50	Metabolite - 3034	<0.0001	<0.0001	0.524	1.03	0.54
12969	61	Metabolite - 3135	<0.0001	1.00E-04	0.197	4.01	0.79
15278	61	Metabolite - 3843	<0.0001	<0.0001	1.547	0.75	1.16
16655	50	Metabolite - 4362	<0.0001	<0.0001	2.185	0.65	1.42

16848	50	Metabolite - 4511	<0.0001	<0.0001	0.460	1.13	0.52
17028	50	Metabolite - 4611	<0.0001	<0.0001	0.782	1.1	0.86
18871	61	Metabolite - 5848	<0.0001	<0.0001	0.380	2.21	0.84
21701	61	Metabolite - 8454	<0.0001	<0.0001	5.638	0.47	2.65
21107	61	5-sulfosalicylate	<0.0001	<0.0001	4.667	0.24	1.12
15686	50	beta-hydroxypyruvate	<0.0001	<0.0001	1.506	0.77	1.16
541	61	4-hydroxyphenylacetate	<0.0001	<0.0001	0.730	1.15	0.84
1303	50	malate	<0.0001	<0.0001	0.407	1.94	0.79
8649	61	Metabolite - 2053	<0.0001	<0.0001	1.407	0.81	1.14
10433	61	Metabolite - 2293	<0.0001	<0.0001	10.000	0.13	1.3
11094	61	Metabolite - 2589	<0.0001	<0.0001	8.727	0.11	0.96
15000	61	Metabolite - 3758	<0.0001	<0.0001	14.091	0.11	1.55
16821	50	Metabolite - 4498	<0.0001	<0.0001	0.613	0.93	0.57
17667	61	Metabolite - 5026	<0.0001	<0.0001	259.000	0.01	2.59
18010	61	Metabolite - 5231	<0.0001	<0.0001	0.425	1.53	0.65
19291	61	Metabolite - 6132	<0.0001	<0.0001	3.920	0.25	0.98
19377	50	Metabolite - 6272	<0.0001	<0.0001	0.532	1.26	0.67
19508	61	Metabolite - 6549	<0.0001	<0.0001	4.781	0.32	1.53
19969	50	Metabolite - 6931	<0.0001	<0.0001	1.605	0.76	1.22
21586	50	Metabolite - 8359	<0.0001	<0.0001	2.200	0.5	1.1
21644	61	Metabolite - 8406	<0.0001	<0.0001	12.917	0.12	1.55
21648	61	Metabolite - 8407	<0.0001	<0.0001	11.636	0.11	1.28
21650	61	Metabolite - 8409	<0.0001	<0.0001	10.500	0.12	1.26
21651	61	Metabolite - 8410	<0.0001	<0.0001	22.600	0.05	1.13
21652	61	Metabolite - 8411	<0.0001	<0.0001	50.400	0.05	2.52
21653	61	Metabolite - 8412	<0.0001	<0.0001	249.000	0.02	4.98
21657	61	Metabolite - 8416	<0.0001	<0.0001	15.000	0.09	1.35
21731	61	Metabolite - 8474	<0.0001	1.00E-04	11.929	0.14	1.67
22880	50	Metabolite - 9286	<0.0001	<0.0001	1.329	0.82	1.09
2150	61	pyridoxamine	1.00E-04	1.00E-04	1.274	0.84	1.07
24285	61	Metabolite - 10026	1.00E-04	2.00E-04	1.333	0.87	1.16
5702	61	Metabolite - 1114	1.00E-04	1.00E-04	0.466	1.16	0.54
21630	50	Metabolite - 8402	1.00E-04	1.00E-04	1.422	0.83	1.18
22590	61	Metabolite - 9040	1.00E-04	2.00E-04	3.783	0.69	2.61
25459	50	Metabolite - 10395	1.00E-04	1.00E-04	0.671	1.4	0.94
10049	61	Metabolite - 2238	1.00E-04	2.00E-04	6.308	0.39	2.46
12109	61	Metabolite - 2853	1.00E-04	1.00E-04	0.475	1.62	0.77
14117	61	Metabolite - 3441	1.00E-04	2.00E-04	0.364	2.64	0.96
16506	50	Metabolite - 4271	1.00E-04	1.00E-04	0.382	1.57	0.6
17151	61	Metabolite - 4656	1.00E-04	1.00E-04	5.833	0.24	1.4
21654	61	Metabolite - 8413	1.00E-04	1.00E-04	>100	0.001	1.92
2832	61	adenosine-5-monophosphate	2.00E-04	2.00E-04	0.291	2.23	0.65
1670	50	urea	4.00E-04	5.00E-04	1.311	0.9	1.18
20769	61	maltotriitol	4.00E-04	4.00E-04	0.454	2.18	0.99
10299	61	Metabolite - 2274	4.00E-04	4.00E-04	6.146	0.41	2.52
63	50	cholesterol	6.00E-04	5.00E-04	1.161	0.93	1.08
1110	50	arachidonic acid	6.00E-04	5.00E-04	0.685	1.27	0.87
19405	50	Metabolite - 6347	6.00E-04	6.00E-04	1.679	0.81	1.36
9016	61	Metabolite - 2109	7.00E-04	6.00E-04	1.695	0.95	1.61
1577	50	2-amino-butyrate	8.00E-04	8.00E-04	1.418	0.91	1.29
12625	50	Metabolite - 3002	8.00E-04	8.00E-04	1.482	0.83	1.23

5800	61	Metabolite - 1188	0.001	9.00E-04	0.271	2.47	0.67
19397	50	Metabolite - 6326	0.001	9.00E-04	1.295	0.95	1.23
12726	50	Metabolite - 3058	0.001	9.00E-04	0.752	1.21	0.91
6161	61	Phthalate-possible	0.0011	0.001	1.781	0.96	1.71
15683	50	4-methyl-2-oxopentanoate	0.0012	0.0011	1.283	0.92	1.18
18232	50	Metabolite - 5403	0.0013	0.0011	1.250	0.92	1.15
18882	61	taurodeoxycholic acid	0.0018	0.0015	0.305	2.46	0.75
8509	61	Metabolite - 2041	0.0018	0.0015	1.140	0.93	1.06
21188	50	1-stearoyl-rac-glycerol	0.0019	0.0016	1.753	0.77	1.35
19490	50	Metabolite - 6488	0.0019	0.0016	2.033	0.6	1.22
12644	50	Metabolite - 3016	0.002	0.0016	1.242	0.91	1.13
25548	50	Metabolite - 10419	0.0021	0.0017	0.865	1.11	0.96
12459	61	Isobar-10-includes-glutamine-H-beta-ala-gly-OH-1-methylguanine-H-Gly-Sar-OH-lysine	0.0028	0.0022	1.168	0.95	1.11
1413	61	3-hydroxyphenylacetate	0.003	0.0024	1.198	0.91	1.09
21047	61	3-methyl-2-oxobutyrate	0.0032	0.0025	1.519	0.81	1.23
16903	61	Metabolite - 4547	0.0033	0.0025	1.505	0.97	1.46
22132	61	DL-alpha-hydroxyisocaproic acid	0.0037	0.0028	0.566	1.22	0.69
21011	50	Metabolite - 7888	0.0039	0.0029	1.508	0.65	0.98
10825	61	Metabolite - 2546	0.0041	0.003	0.565	1.84	1.04
12912	61	Metabolite - 3129	0.0046	0.0033	1.589	0.95	1.51
16893	61	Metabolite - 4530	0.0046	0.0034	0.508	1.3	0.66
599	61	pyruvate	0.0052	0.0037	0.494	1.78	0.88
1604	61	uric acid	0.0054	0.0038	1.095	0.95	1.04
17068	61	Metabolite - 4627	0.0056	0.0039	0.286	2.69	0.77
17614	50	Metabolite - 4966	0.006	0.0042	1.454	0.97	1.41
19934	50	inositol	0.0062	0.0043	0.831	1.18	0.98
12673	50	Metabolite - 3040	0.0063	0.0043	0.592	1.79	1.06
10551	61	Metabolite - 2347	0.007	0.0048	0.235	3.27	0.77
22189	61	palmitoyl-carnitine	0.0076	0.0051	0.725	1.31	0.95
22020	50	Metabolite - 8749	0.0077	0.0052	0.406	2.34	0.95
9491	61	Metabolite - 2185	0.0081	0.0054	1.277	0.94	1.2
22602	50	Metabolite - 9045	0.0097	0.0064	0.673	1.07	0.72
16071	50	Metabolite - 4020	0.0103	0.0067	0.826	1.21	1
15677	61	3-methyl-L-histidine	0.0107	0.007	1.289	0.9	1.16
18476	61	glycocholic acid	0.0107	0.007	0.223	3.37	0.75
16496	50	Metabolite - 4251	0.0122	0.0078	0.798	1.19	0.95
597	61	phosphoenolpyruvate	0.0127	0.008	0.735	1.02	0.75
6851	61	Metabolite - 1497	0.0128	0.008	1.242	0.91	1.13
15650	61	1-methyladenosine	0.015	0.0093	1.117	0.94	1.05
22026	50	1-methylguanidine-hydrochloride	0.0151	0.0093	1.158	0.95	1.1
12774	50	Metabolite - 3094	0.0173	0.0106	0.842	1.14	0.96
7944	61	Metabolite - 1915	0.0176	0.0107	2.514	1.38	3.47
14837	61	Metabolite - 3707	0.0179	0.0108	1.911	1.12	2.14
13589	61	Metabolite - 3327	0.0182	0.0109	0.374	2.97	1.11
9905	61	Metabolite - 2231	0.0183	0.0109	1.195	0.87	1.04

12648	50	Metabolite - 3020	0.0192	0.0115	0.745	1.41	1.05
10604	61	Metabolite - 2370	0.0231	0.0136	0.719	1.35	0.97
17389	50	Metabolite - 4796	0.0238	0.014	0.332	2.65	0.88
14715	61	Stachydrine-possible	0.0257	0.015	0.345	3.54	1.22
1574	61	histamine	0.0264	0.0152	0.841	1.07	0.9
15113	61	Metabolite - 3783	0.0268	0.0153	1.268	0.97	1.23
19514	61	Metabolite - 6553	0.0272	0.0155	1.620	0.5	0.81
20842	61	Metabolite - 7765	0.0288	0.0162	0.513	2.32	1.19
20194	61	Metabolite - 7147	0.0289	0.0162	0.887	1.06	0.94
5577	61	Metabolite - 1065	0.0323	0.0179	2.106	1.41	2.97
15227	61	Metabolite - 3816	0.0323	0.0179	0.767	1.29	0.99
15140	61	L-kynurenine	0.0329	0.0182	1.143	0.98	1.12
9748	61	Metabolite - 2212	0.034	0.0187	0.723	1.41	1.02
5765	61	5-hydroxypentanoate-or-beta-hydroxyisovaleric acid-possible	0.036	0.0197	0.562	2.01	1.13
19372	50	Metabolite - 6269	0.038	0.0207	0.857	0.98	0.84
11111	61	Metabolite - 2592	0.0404	0.0216	2.600	0.9	2.34
20166	61	Metabolite - 7091	0.0415	0.0219	1.443	1.06	1.53
22133	61	DL-hexanoyl-carnitine	0.0429	0.0224	1.240	1	1.24
10629	61	Metabolite - 2386	0.0467	0.0242	1.289	0.83	1.07
15122	50	glycerol	0.048	0.0248	1.175	0.97	1.14
1643	50	fumarate	0.051	0.0262	0.903	1.03	0.93
22337	61	Metabolite - 8893	0.0518	0.0265	1.153	0.98	1.13
8469	61	Metabolite - 2036	0.0521	0.0265	6.559	1.11	7.28
5687	61	Metabolite - 1110	0.0547	0.0274	1.648	1.25	2.06
15500	61	carnitine	0.0591	0.0294	0.821	1.17	0.96
17048	61	Metabolite - 4617	0.0614	0.0303	1.083	0.96	1.04
1105	50	Linoleic acid	0.0647	0.0316	0.882	1.1	0.97
20699	50	meso-erythritol	0.0703	0.0341	0.901	1.11	1
21763	61	Metabolite - 8507	0.0712	0.0342	0.779	1.04	0.81
7941	61	Metabolite - 1914	0.0724	0.0346	1.549	0.82	1.27
1336	50	n-hexadecanoic acid	0.0795	0.0379	0.885	1.13	1
9130	61	Metabolite - 2139	0.0815	0.0386	1.212	0.99	1.2
12720	61	Metabolite - 3056	0.0891	0.0421	0.847	1.18	1
17783	61	trans-2-3-4-trimethoxycinnamic acid	0.0906	0.0425	1.525	0.8	1.22
16226	61	Isobar-28-includes-L-threonine-L-allothreonine-L-homoserine-S-4-amino-2-hydroxybutyric acid	0.0908	0.0425	0.871	1.16	1.01
1358	50	octadecanoic acid	0.099	0.0461	0.916	1.07	0.98
542	50	3-hydroxybutanoic acid	0.1004	0.0466	0.624	1.97	1.23
6266	61	Metabolite - 1286	0.1007	0.0466	1.052	0.97	1.02
18392	61	theobromine	0.1072	0.0494	1.629	1.05	1.71
20950	50	Metabolite - 7846	0.1136	0.0521	0.579	2.35	1.36
18349	61	DL-indole-3-lactic acid	0.1144	0.0522	0.857	1.19	1.02
10245	61	Metabolite - 2269-	0.1162	0.0522	1.487	1.13	1.68
17304	61	Metabolite - 4759	0.1162	0.0522	1.233	0.9	1.11

18034	61	Metabolite - 5234	0.1162	0.0522	1.295	1.12	1.45
22001	61	3-hydroxyoctanoate	0.1208	0.0541	0.773	1.32	1.02
1572	50	glyceric acid	0.126	0.0555	0.874	1.11	0.97
12856	61	Metabolite - 3123	0.1261	0.0555	2.924	0.79	2.31
6497	61	Metabolite - 1374	0.1277	0.056	1.402	1.02	1.43
16650	50	Metabolite - 4360	0.1292	0.0565	0.713	1.29	0.92
10286	61	Metabolite - 2272	0.1333	0.0578	0.625	1.68	1.05
18657	61	Metabolite - 5726	0.1363	0.0588	0.816	1.14	0.93
15681	61	4-Guanidinobutanoic acid	0.1368	0.0588	0.896	1.06	0.95
8176	61	Metabolite - 1974	0.1394	0.0595	1.260	0.96	1.21
21418	61	Isobar-56-includes-DL-pipecolic acid-1-amino-1-cyclopentanecarboxylic acid	0.1529	0.0643	0.768	1.64	1.26
12710	61	Metabolite - 3052	0.1538	0.0644	0.920	1	0.92
13545	61	Metabolite - 3322	0.1626	0.0673	0.730	1.78	1.3
10715	61	Metabolite - 2395	0.163	0.0673	1.527	1.29	1.97
17478	61	Metabolite - 4873	0.1635	0.0673	1.481	0.81	1.2
19097	61	Metabolite - 5969	0.1709	0.0698	1.174	0.92	1.08
18963	61	Metabolite - 5918	0.1718	0.0699	2.337	1.01	2.36
24076	50	Metabolite - 9726	0.1767	0.0714	1.110	1	1.11
17093	61	Metabolite - 4642	0.1812	0.0729	1.146	0.96	1.1
10746	61	Isobar-6-includes-valine-betaine	0.1889	0.0757	1.148	0.88	1.01
6362	61	p-cresol-sulfate	0.1896	0.0758	0.822	1.18	0.97
10092	61	Metabolite - 2250	0.1908	0.0759	0.636	2.06	1.31
22261	61	Isobar-60-includes-s-2-hydroxybutyrate-2-hydroxyisobutyrate	0.1987	0.0785	1.288	0.73	0.94
5733	61	Metabolite - 1127	0.201	0.0792	0.884	1.12	0.99
20830	61	Metabolite - 7762	0.2051	0.0802	1.357	0.98	1.33
10700	61	Metabolite - 2393	0.2053	0.0802	0.928	1.11	1.03
22120	61	6-gamma-gamma-dimethylallyl-amino-purine	0.2065	0.0802	0.951	1.03	0.98
16518	50	Metabolite - 4276	0.2065	0.0802	1.154	0.91	1.05
18829	61	phenylalanine	0.2149	0.0832	0.952	1.05	1
14439	61	Metabolite - 3498	0.2271	0.0876	1.072	0.97	1.04
8300	61	Metabolite - 1988	0.2284	0.0878	1.165	1.03	1.2
6421	61	Metabolite - 1345	0.2299	0.088	1.341	1.23	1.65
1508	61	pantothenic acid	0.2315	0.0884	1.167	1.02	1.19
15121	61	Metabolite - 3786	0.2338	0.0889	0.763	1.18	0.9
18394	61	theophylline	0.2362	0.0893	1.420	1.19	1.69
12626	50	Metabolite - 3003	0.2364	0.0893	0.901	1.01	0.91
6492	61	Metabolite - 1371	0.2409	0.0907	1.369	1.11	1.52
6413	61	phenylacetylglutamine-or-formyl-N-acetyl-5-methoxykynurenamine-possible	0.2441	0.0916	0.836	1.28	1.07
16662	61	Metabolite - 4363	0.2586	0.0967	0.787	1.41	1.11
24077	50	Metabolite - 9727	0.2726	0.1016	0.789	1.47	1.16
22259	61	Isobar-59-includes-N-6-trimethyl-L-lysine-H-	0.281	0.1041	1.106	0.94	1.04

		homoarg-OH					
15676	50	3-methyl-2-oxovaleric acid	0.283	0.1041	0.912	0.91	0.83
13208	61	Metabolite - 3181	0.2918	0.107	0.904	1.04	0.94
10787	61	Metabolite - 2507	0.2989	0.1092	1.308	1.04	1.36
18705	61	Metabolite - 5768	0.3012	0.1097	0.822	1.35	1.11
16865	50	Metabolite - 4522	0.314	0.1133	1.041	0.97	1.01
12756	50	Metabolite - 3077	0.3211	0.1151	1.059	1.01	1.07
16909	61	Metabolite - 4549	0.3323	0.1179	0.716	1.41	1.01
18702	61	Metabolite - 5767	0.3381	0.1196	1.118	0.93	1.04
569	61	caffeine	0.3393	0.1196	0.683	3.82	2.61
1507	50	palmitoleic acid	0.34	0.1196	0.826	1.21	1
20248	61	Metabolite - 7177	0.3483	0.1217	1.317	1.01	1.33
15253	61	Metabolite - 3832-possible-phenol-sulfate	0.3576	0.1246	1.341	1.64	2.2
1645	50	n-dodecanoate	0.3717	0.1291	0.922	1.16	1.07
22577	50	Metabolite - 9035	0.384	0.1329	0.897	1.16	1.04
20267	61	Metabolite - 7187	0.3923	0.1352	1.296	1.15	1.49
7933	61	Metabolite - 1911	0.4027	0.1377	0.702	2.25	1.58
17066	61	Metabolite - 4626	0.4028	0.1377	0.935	1.08	1.01
15529	61	Metabolite - 3951	0.4084	0.1391	0.962	1.05	1.01
513	61	creatinine	0.413	0.1399	0.971	1.04	1.01
8072	61	Metabolite - 1958	0.4193	0.1416	0.971	1.02	0.99
1564	50	citric acid	0.4489	0.1507	0.912	1.13	1.03
15737	50	hydroxyacetic acid	0.4522	0.1514	0.953	1.06	1.01
18015	61	Metabolite - A-3113	0.4749	0.1567	0.879	1.07	0.94
13142	61	Metabolite - 3165	0.4763	0.1567	0.962	1.05	1.01
24233	61	Metabolite - 9855	0.4766	0.1567	0.760	2.04	1.55
15663	61	2-3-dihydroxybenzoic acid	0.4858	0.1583	0.875	1.36	1.19
21421	50	Metabolite - 8214	0.487	0.1583	1.040	1.01	1.05
16070	50	Metabolite - 4019	0.487	0.1583	0.960	1.01	0.97
12478	61	Metabolite - 2898	0.4959	0.16	1.384	1.51	2.09
17271	61	Metabolite - 4746	0.4968	0.16	1.052	0.97	1.02
1417	61	Kynurenic acid	0.5074	0.162	1.066	1.06	1.13
11438	50	phosphate	0.5236	0.1667	0.980	1	0.98
14840	61	Metabolite - 3708	0.5402	0.171	0.887	1.15	1.02
17665	61	p-hydroxybenzaldehyde	0.5451	0.1711	1.020	1	1.02
54	61	tryptophan	0.5459	0.1711	0.981	1.03	1.01
15017	61	Metabolite - 3761	0.5464	0.1711	0.934	1.06	0.99
13179	61	Metabolite - 3176-possible-creatine	0.5563	0.1732	0.936	1.1	1.03
14961	61	Metabolite - 3752	0.5662	0.1758	1.064	0.94	1
17298	61	Metabolite - 4756	0.571	0.1768	1.071	0.98	1.05
22053	61	3-hydroxydecanoic acid	0.5741	0.1773	0.917	1.08	0.99
10317	61	Metabolite - 2279	0.5777	0.1779	0.849	1.39	1.18
7029	61	Metabolite - 1597	0.5816	0.1786	1.020	1	1.02
16244	61	Isobar-21-includes-gamma-aminobutyryl-L-histidine-L-anserine	0.5964	0.1816	0.952	1.05	1
21044	50	Metabolite - s-2-hydroxybutyric acid	0.6118	0.1857	1.064	1.09	1.16

10501	61	Metabolite - 2321	0.6132	0.1857	1.099	1.21	1.33
19787	61	Metabolite - 6746	0.62	0.187	0.981	1.05	1.03
1301	50	lysine	0.6207	0.187	0.922	1.29	1.19
16939	61	Metabolite - 4586	0.6303	0.1889	1.032	0.93	0.96
19906	61	Metabolite - 6827	0.6345	0.1896	1.071	1.12	1.2
10304	61	Metabolite - 2276	0.6399	0.1897	1.164	1.34	1.56
22145	61	O-acetyl-L-carnitine-hydrochloride	0.6448	0.19	1.029	1.04	1.07
5809	61	3-indoxyl-sulfate	0.646	0.19	0.936	1.1	1.03
18706	61	Metabolite - 5769	0.6599	0.1935	0.963	1.08	1.04
12604	50	Metabolite - 2981	0.6621	0.1936	1.020	0.99	1.01
17488	61	Metabolite - 4887	0.6822	0.1975	0.966	0.89	0.86
1299	61	tyrosine	0.7135	0.2055	1.020	1.01	1.03
22154	61	bradykinin	0.7224	0.2069	0.925	1.46	1.35
606	61	uridine	0.7239	0.2069	1.020	1.01	1.03
12035	50	nonanate	0.7322	0.2087	0.990	0.99	0.98
6144	61	Metabolite - 1215	0.7562	0.2145	0.861	3.88	3.34
21762	61	Metabolite - 8506	0.785	0.222	0.939	1.65	1.55
1506	61	orotidine-5-phosphate	0.789	0.2226	0.971	1.03	1
13038	61	Metabolite - 3143	0.7911	0.2227	0.943	1.23	1.16
2734	61	gamma-L-glutamyl-L-tyrosine	0.8158	0.2262	1.021	0.97	0.99
12924	61	Metabolite - 3131	0.8273	0.2288	1.033	1.22	1.26
1642	50	decanoic acid	0.8396	0.2305	1.022	0.93	0.95
22895	50	Metabolite - 9299	0.8504	0.2323	1.023	0.86	0.88
16016	61	Metabolite - 3994	0.8777	0.2388	1.024	0.84	0.86
15255	61	Metabolite - 3833	0.8785	0.2388	1.040	1.24	1.29
13146	61	Metabolite - 3166	0.8821	0.2392	0.970	0.99	0.96
17033	61	Metabolite - 4613	0.8939	0.2412	1.021	0.95	0.97
15753	61	hippuric acid	0.9065	0.2441	1.022	1.38	1.41
15612	61	Metabolite - 3972	0.9499	0.2546	1.000	0.97	0.97
18254	61	1-7-dimethylxanthine	0.9503	0.2546	1.026	1.94	1.99
594	61	niacinamide	0.9552	0.2548	1.024	0.83	0.85
15326	61	Metabolite - 3879	0.9567	0.2548	0.985	1.31	1.29
15765	61	ethylmalonic acid	0.9909	0.2611	1.000	0.96	0.96
1570	50	oleic acid	0.9978	0.2623	1.000	1.03	1.03

3E: Algorithms (models) for diagnosing Metabolic Syndrome and pre-disposition to Metabolic Syndrome (insulin sensitivity).

[00222] Models were developed to test the ability to predict insulin sensitivity (Rd) and metabolic syndrome using the biomarker metabolites alone and/or in combination with clinical measures of metabolic syndrome (e.g. BMI, Rd). The plasma samples used for the analysis were from subjects with various rates of glucose disposal (Rd).

[00223] Algorithms for determining insulin sensitivity were developed by multiple iterations of regression analysis of glucose utilization rate (i.e. Rd) in combination with

measurements of metabolite biomarkers. The samples were divided into two groups. The first group was used as a 'training' set and the second group was used as a 'test' set. Then a model was developed using the training set and the predictive power of the resulting model was determined using the test set. Several models were developed to identify the most important biomarker metabolites for predicting insulin sensitivity and thereby demonstrating the utility of this approach and these biomarker metabolites.

[00224] A model was developed to predict insulin sensitivity (i.e. Rd) using plasma samples collected from a cohort with varying levels of insulin sensitivity and BMI less than 27.9 and BMI greater than 27.9. For this model the training group included half of the plasma samples and was balanced for BMI and Rd. The model was then tested using a test group that included the other half of the samples and was also balanced for BMI and Rd. The results of this analysis showed that the best model for predicting insulin sensitivity includes: BMI and the biomarker metabolites glucose, 3-methyl-2-oxobutyric acid, 1,5-anhydroglucitol and metabolite-6268.

[00225] Another model was developed to predict insulin sensitivity (i.e. Rd) using plasma samples collected from a cohort with varying levels of insulin sensitivity and BMI less than 27.9. For this model the training group included half of the plasma samples and was balanced for insulin sensitivity (Rd). The model was then tested using a test group that included the other half of the plasma samples and was also balanced for Rd. The results of this analysis showed that the best model for predicting insulin sensitivity includes the biomarker metabolites: glucose, metabolite-2546, metabolite-2853, metabolite-2370 and metabolite-2386.

[00226] Yet another model was developed to predict insulin sensitivity (i.e. Rd) using plasma samples collected from a cohort with varying levels of insulin sensitivity and BMI greater than 27.9. For this model the training group included half of the plasma samples and was balanced for insulin sensitivity (Rd). The model was then tested using a test group that included the other half of the plasma samples and was also balanced for Rd. The results of this analysis showed that the best model for predicting insulin sensitivity includes the biomarker metabolites: 3-methyl-2-oxobutyric, metabolite-3097, metabolite-4020, metabolite -3056 and metabolite-1831.

[00227] The model: BMI and the biomarker metabolites glucose, 3-methyl-2-

oxobutyric acid, 1,5-anhydroglucitol and metabolite-6268; was used on a new cohort to predict insulin sensitivity (Rd). The model was developed to predict insulin sensitivity (i.e. Rd) using plasma samples collected from a cohort with varying levels of insulin sensitivity and BMI less than 27.9 and BMI greater than 27.9, as described above. The samples used to test the model for this analysis were obtained from 19 Caucasian males aged 18-39, average age of 25.6, that had been diagnosed with metabolic syndrome and 19 healthy, age-matched, Caucasian males. Plasma samples and serum samples were evaluated. The results of this analysis show that the model could correctly predict insulin sensitivity in this new cohort using either plasma (Figure 21) or serum (Figure 22) samples.

Example 4: Treatment Response Biomarkers

[00228] Biomarkers that are predictive of response to treatment were identified through comparisons of subjects that were 'non-responders' (i.e. those with little or no change (<15%) in Rd between baseline and 12 weeks post-treatment) and those subjects that were responsive to the treatment (i.e. 'responders'). Biomarkers that were predictive of subjects as treatment responders or non-responders were based on compound levels at baseline only. The responders were defined either as those subjects with either a Rd change of 35% or higher or as those with a Rd change of 15% or higher. Data was analyzed by comparing Non-responders with both classes of Responders. Both analyses (i.e. Non-responder with Rd change under 15% vs Responder with Rd change over 35%, Non-responder with Rd change under 15% vs Responder with Rd change over 15%) were then combined and those biomarkers with a p value of <0.1 in EITHER of the 2 analyses were identified. The biomarkers are listed in Table 27. The biomarker measurements before treatment were predictive of thiazolidinedione (TZD) response, and thus can be used to select patients for treatment with TZD drugs. Experiments are planned to evaluate the biomarkers as predictive to other treatments for insulin sensitivity, pre-diabetes and diabetes control such as other therapeutic agents (e.g. metformin, etc.), weight loss, nutrition and other lifestyle modifications. This group of predictive biomarkers provide an extremely valuable tool for personalized medicine.

Table 27. Biomarkers to Classify Responders or Non-Responders of Treatment

COMPOUND	LIB_ ID	COMP_ ID	P value (Responder vs Non-Responder)
Metabolite - 11737	200	33082	0.0007
Metabolite - 11849	201	33194	0.0013
inositol	50	19934	0.0041
glycerophosphorylcholine (GPC)	200	15990	0.0043
phenylalanine	200	64	0.0045
acetylcarnitine	200	32198	0.0049
linoleate (18:2(n-6))	201	32673	0.0079
Metabolite - 11845	201	33190	0.0098
tryptophan	200	54	0.0107
Metabolite - 10407	50	25522	0.0113
Metabolite - 11379	201	32696	0.0117
Metabolite - 9727	50	24077	0.0136
Metabolite - 11205	200	32519	0.0151
Metabolite - 11883	200	33228	0.0168
Metabolite - 10954_200	200	32734	0.0178
glycerol	50	15122	0.0182
gondoate-20-1-n-9-	201	32402	0.0182
Metabolite - 03832_201	201	32553	0.0209
oleate (18:1(n-9))	201	32630	0.0225
Metabolite - 11793	200	33138	0.0225
gamma-glutamylphenylalanine-	200	33362	0.0231
Metabolite - 11560	201	32877	0.0242
Metabolite - 11247	201	32564	0.0273
Metabolite - 11887	201	33232	0.0317
Metabolite - 11206	200	32520	0.0317
tyrosine	200	1299	0.0317
Metabolite - 12064	201	33416	0.0317
EDTA*	201	32511	0.0317
Metabolite - 11790	200	33135	0.0338
3-hydroxybutyrate (BHBA)	50	542	0.0339
palmitoleate (16:1(n-7))	201	32628	0.0412
lysine	50	1301	0.0468
Metabolite - 11314	200	32631	0.0468
Metabolite - 11204	200	32518	0.0468
Metabolite - 11437	201	32754	0.0491
alpha linolenate (18:3(n-3))	201	32416	0.0499
peptide-DSGEGDFXAEGGGVR	200	31548	0.0531
Metabolite - 11874	201	33219	0.0531
palmitate (16:0)	201	1336	0.0547
methionine	201	1302	0.0566
glycerol 3-phosphate (G3P)	50	15365	0.0566
Metabolite - 2800	50	16287	0.0593
Metabolite - 11522	201	32839	0.0611
Metabolite - 11421	200	32738	0.062
Metabolite - 11881	201	33226	0.0637
4-methyl-2-oxopentanoate	201	22116	0.0637

quinate	50	18335	0.0649
pelargonate-9-0-	201	12035	0.0676
creatinine	200	513	0.0676
3-methyl-2-oxobutyrate	201	21047	0.0676
Metabolite - 11235	201	32552	0.0692
Metabolite - 6272	50	19377	0.071
Metabolite - 10360	50	25402	0.0726
saccharin	201	21151	0.0734
Metabolite - 11593	201	32910	0.0742
ornithine	50	1493	0.076
cholate	201	22842	0.0775
1,6-anhydroglucose	50	21049	0.0787
Metabolite - 11435	201	32752	0.0829
Metabolite - 11832	201	33177	0.0891
deoxycholate	201	1114	0.0902
Metabolite - 11880	201	33225	0.0902
docosahexaenoate (DHA)	50	19323	0.0927
Metabolite - 12056	200	33408	0.0981
Metabolite - 3075	50	12754	0.0987

[00229] Recursive partioning analysis was carried out on the subjects. Baseline levels (i.e. prior to treatment) of the biomarker compounds were determined in the Responders (subjects with a post-treatment increase in Rd $\geq$ 35%, N=28) and the Non-Responders (subjects with a post-treatment Rd increase of <15%, N=14). The results of this analysis showed that the subjects were classified with an AUC of 0.8214. The analysis further identified "Metabolite – 11737" as a biomarker that is particularly important in the classification of responders and non-responders. Using the baseline level of only this biomarker, 22 of 28 Responders were correctly classified and 12 of 14 Non-Responders were correctly classified. This marker alone had a sensitivity of 78.6% and a specificity of 85.7%. The Positive Predictive Value (PPV) was 91.7% and the Negative Predictive Value (NPV) was 66.7%.

[00230] Biomarkers that are pharmacodynamic (PD) biomarkers of treatment effectiveness were identified through comparisons of non-responders (i.e. those with little or no change (<15%) in Rd between baseline and 12 weeks post-treatment) vs responders. The PD biomarkers were based on the difference between baseline and 12 weeks post-treatment. Biomarkers were identified that showed changes from baseline levels upon TZD treatment and tracked with the change in insulin sensitivity in those subjects. These biomarkers are listed in Table 28.

Table 28 Pharmacodynamic Biomarkers of Treatment Response

COMPOUND	COMP_ID	LIB_ID	P-Value (Responder vs Non-Responder)
Metabolite - 11737	33082	200	0.0007
Metabolite - 11849	33194	201	0.0013
palmitoylglycerol (monopalmitin)	21127	50	0.0041
glutamate	12751	50	0.0041
glycerophosphorylcholine (GPC)	15990	200	0.0043
acetylcarnitine	32198	200	0.0049
gamma-glutamylphenylalanine	33362	200	0.0052
alpha-tocopherol	1561	50	0.0053
glucose	20488	50	0.0062
phenylalanine	64	200	0.0071
inositol	19934	50	0.0075
Metabolite - 9727	24077	50	0.0079
Metabolite - 11385	32702	200	0.0083
Metabolite - 11845	33190	201	0.0098
tryptophan	54	200	0.0107
Metabolite - 10407	25522	50	0.0113
erythritol	20699	50	0.0136
Metabolite - 11205	32519	200	0.0151
Metabolite - 11883	33228	200	0.0168
Metabolite - 10954	32734	200	0.0178
methionine	1302	201	0.0196
Metabolite - 03832	32553	201	0.0209
5-oxoproline	1494	200	0.0242
Metabolite - 4611	17028	50	0.0242
linoleate (18:2(n-6))	32673	201	0.0258
galactonic acid	27719	50	0.0259
Metabolite - 11247	32564	201	0.0273
dipalmitin	27392	50	0.0297
tyrosine	1299	200	0.0317
Metabolite - 11379	32696	201	0.035
Metabolite - 11560	32877	201	0.0387
Metabolite - 11437	32754	201	0.0491
Metabolite - 11206	32520	200	0.0492
Metabolite - 11475	32792	201	0.0564
Metabolite - 11254	32571	200	0.0564
Metabolite - 10610	27889	50	0.0564
glycerol 3-phosphate (G3P)	15365	50	0.0566
gondoate-20-1-n-9	32402	201	0.0566
Metabolite - 2800	16287	50	0.0593
Metabolite - 11386	32703	200	0.06
Metabolite - 4055	16120	50	0.06
Metabolite - 11421	32738	200	0.062
Metabolite - 11244	32561	201	0.0637
Metabolite - 6486	19487	50	0.0637
Metabolite - 7846	20950	50	0.0637
quinate	18335	50	0.0649

Metabolite - 11881	33226	201	0.0649
3-methyl-2-oxovalerate	15676	201	0.0676
Metabolite - 9045	22602	50	0.0676
Metabolite - 4360	16650	50	0.0676
Metabolite - 11788	33133	200	0.0676
Metabolite - 6272	19377	50	0.071
3-hydroxybutyrate (BHBA)	542	50	0.071
oleate (18:1(n-9))	32630	201	0.071
Metabolite - 10360	25402	50	0.0726
saccharin	21151	201	0.0734
Metabolite - 11593	32910	201	0.0742
Metabolite - 11790	33135	200	0.0749
kynurenine	15140	200	0.076
Metabolite - 4357	16634	50	0.076
Metabolite - 3100	12782	50	0.076
cholate	22842	201	0.0775
1,6-anhydroglucose	21049	50	0.0787
Metabolite - 11887	33232	201	0.081
glycerol	15122	50	0.081
Metabolite - 06126	32557	201	0.0829
Metabolite - 4986	17627	50	0.0853
Metabolite - 3099	12781	50	0.0902
Metabolite - 11314	32631	200	0.0921
docosahexaenoate (DHA)	19323	50	0.0927
Metabolite - 11522	32839	201	0.0954
Metabolite - 01142	32747	201	0.0954
Metabolite - 3075	12754	50	0.0987

[00231] Recursive partioning analysis was carried out on the subjects. Baseline levels (i.e. prior to treatment) and post-treatment levels of the biomarker compounds were determined in the Responders (subjects with a post-treatment increase in Rd $\geq$ 35%, N=28) and the Non-Responders (subjects with a post-treatment Rd increase of <15%, N=14). The results of this analysis showed that the subjects were classified with an AUC of 0.7679. Additional experiments are planned to evaluate the biomarkers as PD biomarkers for other insulin sensitivity, pre-diabetes and diabetes therapeutic agents (e.g. metformin, etc.) as well as treatments involving modification to diet (e.g. weight loss, nutrition) and lifestyle (e.g. exercise).

Example 5: Analytical characterization of unnamed biomarkers compounds:

[00232] Table 29 below includes analytical characteristics of each of the unnamed metabolites listed in the Tables above. The table includes, for each listed Metabolite, the

retention time (RT), retention index (RI), mass, quant mass, and polarity obtained using the analytical methods described above. "Mass" refers to the mass of the C12 isotope of the parent ion used in quantification of the compound. "Polarity" indicates the polarity of the quantitative ion as being either positive (+) or negative (-).

Table 29. Analytical characteristics of biomarker metabolites.

COMP_ID	COMPOUND	LIBRARY	RT	RI	MASS	POLARITY
25532	Metabolite - 10413	50	12.53	2042.7	204.1	+L
25602	Metabolite - 10432	50	12.29	2031.5	204	+L
27256	Metabolite - 10500	50	5.3	1229.9	211	+L
27264	Metabolite - 10503	50	7.28	1452.4	244	+L
27889	Metabolite - 10610	50	11.93	1987	204	+L
30288	Metabolite - 10750	50	5.51	1265	102.9	+L
30290	Metabolite - 10752	50	6.07	1323.7	231	+L
30832	Metabolite - 10814	50	12.84	2094	204.1	+L
31373	Metabolite - 10878	50	8.22	1583	334.1	+L
31509	Metabolite - 10931	50	12.02	1984	174.1	+L
31518	Metabolite - 10933	50	11.99	1979	318.1	+L
12781	Metabolite - 3099	50	11.77	2002.3	204	+L
12782	Metabolite - 3100	50	11.85	2010.7	204	+L
12795	Metabolite - 3113	50	12.73	2111.4	406.2	+L
16120	Metabolite - 4055	50	12.04	2021.4	304.1	+L
16138	Metabolite - 4080 - retired for 1-palmitoyl-sn-glycero-3-phosphocholine	50	14.02	2266.9	299	+L
16509	Metabolite - 4273	50	10.34	1844.2	457.2	+L
16511	Metabolite - 4274	50	10.37	1854.7	158.1	+L
16512	Metabolite - 4275 - retired - part of X-3078	50	10.68	1884.6	345.2	+L
16518	Metabolite - 4276 - retired for gamma-tocopherol*	50	13.92	2261	223.1	+L
16634	Metabolite - 4357	50	8	1540.5	216	+L
16650	Metabolite - 4360	50	9.15	1678.4	347.2	+L
16665	Metabolite - 4364 - retired for L-asparagine - 3	50	10.66	1852.1	232	+L
16666	Metabolite - 4365	50	11.05	1893.1	204	+L
16829	Metabolite - 4503	50	8.39	1589.3	227.2	+L
17028	Metabolite - 4611 - retired for erythronic acid *	50	8.07	1546.9	292.1	+L
17330	Metabolite - 4769 retired for glutamine -	50	11.3	1916.6	156	+L

	2					
17389	Metabolite - 4796	50	3.53	1043.2	117	+L
17690	Metabolite - 5207	50	7.41	1493.6	151	+L
18120	Metabolite - 5348	50	9.25	1681.5	217.9	+L
19462	Metabolite - 6446	50	3.49	1021.1	204.1	+L
19478	Metabolite - 6467	50	11.09	1893.4	320.1	+L
19487	Metabolite - 6486	50	11.6	1949.8	217	+L
19576	Metabolite - 6627	50	11.96	1990.7	304.2	+L
19983	Metabolite - 6955	50	11.82	1979.1	306.1	+L
12162	Metabolite - A-2339	50	3.86	1109.8	221.0	+L
12222	Metabolite - A-2374	50	7.35	1510.9	188.0	+L
12803	Metabolite - A-2441	50	13.94	2270.9	129.0	+L
16074	Metabolite - A-2758	50	8.22	1597.1	211.0	+L
16285	Metabolite - A-2798	50	3.44	1005.8	163.0	+L
16287	Metabolite - A2800	50	3.53	1015.5	191.1	+L
24360	Metabolite -10206	50	9.04	1639.0	243.0	+L
25402	Metabolite -10360	50	10.23	1780.0	204.0	+L
25429	Metabolite -10369	50	10.92	1859.0	333.0	+L
25459	Metabolite -10395	50	9.94	1768.9	156.0	+L
25522	Metabolite -10407	50	9.94	1748.0	217.1	+L
25548	Metabolite -10419	50	16.29	2527.0	311.3	+L
25584	Metabolite -10425	50	7.52	1488.8	123.9	+L
25597	Metabolite -10427	50	11.21	1911.1	183.0	+L
25598	Metabolite -10428	50	11.31	1922.0	156.0	+L
25599	Metabolite -10429	50	11.60	1953.5	265.0	+L
25607	Metabolite -10437	50	8.43	1596.0	331.1	+L
25609	Metabolite -10439	50	8.84	1643.3	331.1	+L
25649	Metabolite -10450	50	17.00	2643.0	371.3	+L
27137	Metabolite -10498	50	12.06	1991.8	299.1	+L
27271	Metabolite -10504	50	9.94	1763.0	348.2	+L
27272	Metabolite -10505	50	10.82	1862.0	457.3	+L
27273	Metabolite -10506	50	11.30	1914.0	218.1	+L
27275	Metabolite -10507	50	11.97	1988.0	370.2	+L
27278	Metabolite -10510	50	15.77	2470.0	297.2	+L
27279	Metabolite -10511	50	17.12	2645.0	309.3	+L
27288	Metabolite -10517	50	10.16	1775.0	419.2	+L
27326	Metabolite -10527	50	11.71	1950.0	221.1	+L
27678	Metabolite -10584 - retired for glucose-3	50	10.15	1779.0	217.0	+L
27841	Metabolite -10595	50	4.14	1101.0	151.0	+L
27888	Metabolite -10609	50	11.70	1961.0	348.2	+L
27890	Metabolite -10611	50	12.03	1998.0	369.1	+L
28059	Metabolite -10650	50	10.26	1800.6	345.1	+L

29817	Metabolite -10683	50	5.12	1213.8	171.0	+L
30265	Metabolite -10732	50	12.22	2024.0	204.0	+L
30273	Metabolite -10736	50	10.03	1814.0	342.1	+L
30282	Metabolite -10744	50	15.75	2503.0	311.2	+L
12533	Metabolite -2915	50	3.77	1099.0	174.0	+L
12593	Metabolite -2973	50	4.74	1213.4	281.0	+L
12604	Metabolite -2981	50	5.21	1265.2	211.0	+L
12609	Metabolite -2986	50	5.56	1304.3	201.0	+L
12625	Metabolite -3002	50	6.74	1440.8	296.0	+L
12626	Metabolite -3003	50	6.79	1446.6	218.0	+L
12638	Metabolite -3011	50	7.08	1479.2	174.0	+L
12639	Metabolite -3012	50	7.17	1489.8	232.0	+L
12644	Metabolite -3016	50	7.58	1537.5	186.0	+L
12645	Metabolite -3017	50	7.61	1541.4	246.0	+L
12647	Metabolite -3019	50	7.74	1556.4	260.0	+L
12648	Metabolite -3020	50	7.81	1564.1	292.0	+L
12650	Metabolite -3022	50	7.98	1584.9	142.0	+L
12656	Metabolite -3025	50	8.11	1600.3	274.0	+L
12658	Metabolite -3026	50	8.17	1606.1	274.0	+L
12663	Metabolite -3030	50	8.62	1659.7	320.0	+L
12666	Metabolite -3033	50	8.88	1689.4	117.0	+L
12667	Metabolite -3034	50	8.92	1694.9	299.0	+L
12673	Metabolite -3040	50	9.27	1735.7	274.0	+L
12726	Metabolite -3058	50	9.70	1786.9	335.0	+L
12742	Metabolite -3067	50	10.02	1824.2	132.0	+L
12751	Metabolite -3073	50	10.17	1838.8	362.0	+L
12753	Metabolite -3074	50	10.22	1844.5	204.0	+L
12754	Metabolite -3075	50	10.36	1857.9	204.0	+L
12756	Metabolite -3077	50	10.44	1866.2	308.0	+L
12757	Metabolite -3078	50	10.65	1887.0	203.0	+L
12767	Metabolite -3087	50	11.19	1942.0	174.0	+L
12768	Metabolite -3088	50	11.23	1946.1	372.0	+L
12770	Metabolite -3090	50	11.31	1955.0	243.0	+L
12771	Metabolite -3091	50	11.41	1966.2	232.0	+L
12773	Metabolite -3093	50	11.50	1975.6	204.0	+L
12774	Metabolite -3094	50	11.55	1980.6	299.0	+L
12777	Metabolite -3097	50	11.64	1990.4	204.0	+L
12780	Metabolite -3098	50	11.75	2003.0	308.0	+L
12783	Metabolite -3101	50	11.93	2022.2	290.0	+L
12785	Metabolite -3103	50	12.09	2039.8	290.0	+L
12789	Metabolite -3107	50	12.21	2053.2	204.0	+L
12790	Metabolite -3108	50	12.24	2056.5	246.0	+L

12791	Metabolite -3109	50	12.56	2092.6	202.0	+L
12796	Metabolite -3114	50	12.79	2120.6	204.0	+L
16028	Metabolite -3998	50	5.22	1252.7	171.0	+L
16044	Metabolite -4005	50	6.53	1401.3	86.0	+L
16060	Metabolite -4014	50	7.17	1474.9	252.0	+L
16070	Metabolite -4019	50	7.68	1534.5	174.0	+L
16071	Metabolite -4020	50	7.91	1561.5	220.0	+L
16116	Metabolite -4051	50	11.56	1970.2	357.0	+L
16290	Metabolite -4133	50	4.35	1108.9	198.0	+L
16308	Metabolite -4147	50	10.07	1767.1	290.0	+L
16496	Metabolite -4251	50	4.09	1130.7	217.0	+L
16506	Metabolite -4271	50	9.69	1777.4	419.0	+L
16653	Metabolite -4361	50	9.40	1706.2	232.0	+L
16655	Metabolite -4362	50	10.02	1779.9	319.0	+L
16819	Metabolite -4496	50	6.76	1398.2	204.0	+L
16821	Metabolite -4498	50	7.06	1434.9	103.0	+L
16831	Metabolite -4504	50	8.46	1597.1	244.0	+L
16843	Metabolite -4510	50	9.70	1740.1	254.0	+L
16848	Metabolite -4511	50	10.09	1788.4	206.0	+L
16859	Metabolite -4516	50	11.00	1886.5	217.0	+L
16860	Metabolite -4517	50	11.06	1892.7	217.0	+L
16865	Metabolite -4522	50	12.26	2025.4	217.0	+L
16983	Metabolite -4598	50	6.69	1392.2	170.0	+L
16984	Metabolite -4599	50	7.42	1471.1	113.0	+L
17064	Metabolite -4624	50	10.01	1779.1	342.0	+L
17083	Metabolite -4634	50	11.00	1884.3	333.0	+L
17327	Metabolite -4767	50	8.77	1626.2	117.0	+L
17359	Metabolite -4791	50	10.29	1796.4	366.5	+L
17390	Metabolite -4806	50	4.20	1122.8	105.0	+L
17614	Metabolite -4966	50	9.66	1749.4	218.0	+L
17627	Metabolite -4986	50	11.56	1956.4	204.0	+L
17971	Metabolite -5210	50	8.47	1616.4	254.0	+L
17975	Metabolite -5211	50	8.77	1652.1	326.0	+L
17978	Metabolite -5213	50	8.97	1675.3	111.0	+L
17987	Metabolite -5228	50	6.97	1442.5	181.0	+L
18118	Metabolite -5346	50	8.33	1573.0	202.0	+L
18122	Metabolite -5349	50	10.10	1782.2	312.0	+L
18146	Metabolite -5366	50	12.49	2044.7	204.0	+L
18147	Metabolite -5367	50	12.77	2079.3	171.0	+L
18232	Metabolite -5403	50	5.92	1300.2	319.0	+L
18273	Metabolite -5420	50	9.09	1669.0	417.0	+L
18384	Metabolite -5487	50	7.01	1426.3	204.0	+L

18388	Metabolite -5491	50	8.30	1575.9	129.0	+L
18868	Metabolite -5847	50	12.35	2040.0	288.2	+L
18929	Metabolite -5907	50	8.69	1643.2	229.1	+L
19110	Metabolite -5978	50	7.52	1468.9	232.1	+L
19362	Metabolite -6226	50	4.38	1137.4	154.0	+L
19363	Metabolite -6227	50	5.00	1210.5	196.1	+L
19364	Metabolite -6246	50	6.94	1428.2	160.1	+L
19367	Metabolite -6266	50	9.15	1683.5	240.2	+L
19368	Metabolite -6267	50	9.32	1704.5	257.1	+L
19370	Metabolite -6268	50	9.91	1773.8	271.1	+L
19372	Metabolite -6269 - retired for inositol-3	50	10.88	1880.9	217.1	+L
19374	Metabolite -6270	50	11.35	1929.6	320.2	+L
19377	Metabolite -6272	50	12.60	2069.6	131.0	+L
19383	Metabolite -6286	50	16.36	2553.7	311.3	+L
19397	Metabolite -6326	50	7.66	1510.9	144.1	+L
19402	Metabolite -6346	50	8.00	1550.8	263.2	+L
19405	Metabolite -6347	50	8.16	1568.7	244.1	+L
19414	Metabolite -6350	50	11.41	1937.2	169.0	+L
19490	Metabolite -6488	50	12.25	2021.7	204.1	+L
19494	Metabolite -6506	50	12.81	2084.7	204.1	+L
19596	Metabolite -6647	50	9.13	1696.7	197.1	+L
19597	Metabolite -6648	50	9.17	1702.1	313.2	+L
19599	Metabolite -6649	50	11.47	1955.4	299.2	+L
19623	Metabolite -6671	50	9.65	1738.4	229.0	+L
19955	Metabolite -6907	50	9.22	1686.9	337.1	+L
19961	Metabolite -6913	50	9.53	1726.0	171.0	+L
19968	Metabolite -6930	50	10.32	1809.4	331.2	+L
19969	Metabolite -6931	50	10.35	1819.6	267.1	+L
20299	Metabolite -7266	50	7.82	1517.6	115.0	+L
20950	Metabolite -7846	50	5.10	1208.1	145.1	+L
21011	Metabolite -7888	50	15.96	2513.3	311.3	+L
21012	Metabolite -7889	50	16.83	2629.4	311.3	+L
21013	Metabolite -7890	50	17.76	2752.2	129.0	+L
21415	Metabolite -8209	50	14.77	2338.0	456.5	+L
21421	Metabolite -8214	50	17.13	2646.6	311.2	+L
21586	Metabolite -8359	50	7.14	1457.8	253.0	+L
21630	Metabolite -8402	50	15.27	2424.0	283.1	+L
21631	Metabolite -8403	50	15.96	2516.6	309.2	+L
22020	Metabolite -8749 - retired for fructose-4	50	9.74	1763.0	204.1	+L
22032	Metabolite -8766	50	12.22	2034.0	315.1	+L
22054	Metabolite -8792	50	17.61	2737.0	129.0	+L
22320	Metabolite -8889	50	8.62	1635.0	521.2	+L

22480	Metabolite -8987	50	7.02	1449.7	160.1	+L
22494	Metabolite -8994	50	10.76	1879.0	447.2	+L
22507	Metabolite -9010 - retired for adenosine - 1	50	12.98	2126.2	217.1	+L
22548	Metabolite -9026	50	8.45	1600.0	156.0	+L
22555	Metabolite -9027	50	8.50	1605.0	357.2	+L
22570	Metabolite -9033	50	9.61	1736.4	217.1	+L
22572	Metabolite -9034	50	9.63	1739.1	241.1	+L
22577	Metabolite -9035	50	9.82	1760.7	285.1	+L
22600	Metabolite -9043	50	11.75	1974.1	204.1	+L
22601	Metabolite -9044	50	13.38	2169.0	204.1	+L
22602	Metabolite -9045	50	13.91	2239.0	450.3	+L
22609	Metabolite -9047	50	8.06	1574.0	243.2	+L
22649	Metabolite -9108	50	11.20	1896.0	156.0	+L
22880	Metabolite -9286	50	8.77	1617.5	221.0	+L
22895	Metabolite -9299	50	10.54	1827.5	305.1	+L
22993	Metabolite -9448	50	14.86	2352.5	343.2	+L
24074	Metabolite -9706	50	4.39	1107.0	190.0	+L
24076	Metabolite -9726	50	4.91	1167.0	245.0	+L
24077	Metabolite -9727	50	5.24	1204.0	177.0	+L
10737	Isobar 01	61	1.45	1481.0	225.0	-i
10743	Isobar 04	61	1.52	1567.0	195.0	-i
10746	Isobar 06	61	2.13	2160.0	118.0	+i
10750	Isobar 08	61	10.04	10116.0	138.0	+i
12459	Isobar 10	61	1.40	1527.0	147.0	+i
16233	Isobar 13	61	1.40	1530.0	193.0	-i
16232	Isobar 17	61	1.49	1620.0	175.0	+i
16235	Isobar 19	61	1.55	1700	199	-i
16244	Isobar 21	61	1.59	1620.0	241.0	+i
16228	Isobar 22	61	1.55	1635.0	148.0	+i
16229	Isobar 24	61	1.43	1545.0	153.0	+i
16226	Isobar 28	61	1.46	1525.0	120.0	+i
18829	Isobar 45	61	8.38	8475.0	166.2	+i
18882	Isobar 47	61	15.51	15700.0	498.4	-i
21404	Isobar 48	61	1.50	1550.0	106.1	+i
21410	Isobar 52	61	1.55	1650.0	134.1	+i
21418	Isobar 56	61	2.45	2850.0	130.1	+i
22258	Isobar 58	61	1.38	1620.0	164.0	+i
22259	Isobar 59	61	1.82	1700.0	189.1	+i
22261	Isobar 60	61	4.26	4725.0	148.9	-i
22262	Isobar 61	61	9.30	9675.0	174.8	-i
22803	Isobar 66	61	15.06	15500.0	450.2	+i

27773	Isobar 71	61	1.57	1700.0	206.9	-i
32718	Metabolite - 01342_200	200	2.8	2848	265.1	+i
32735	Metabolite - 01911_200	200	4.26	4275	464.1	+i
32596	Metabolite - 02250_200 - retired for piperine	200	5.14	5158	286.2	+i
32672	Metabolite - 02546_200	200	0.75	764	129.2	+i
32829	Metabolite - 03653_200 - retired for stachydrine	200	0.82	826	144.2	+i
32595	Metabolite - 08893_200	200	5.19	5200	431.9	+i
32734	Metabolite - 10954_200 - retired for (+/-) octanoyl carnitine	200	4.14	4229	288.2	+i
32514	Metabolite - 11200 - retired for 1-palmitoyl-sn-glycero-3-phosphocholine	200	5.62	5637	496.4	+i
32516	Metabolite - 11202 - retired for 1-stearoyl-sn-glycero-3-phosphocholine	200	5.8	5823	524.4	+i
32517	Metabolite - 11203 - retired for 1-Oleoyl-sn-glycero-3-phosphocholine	200	5.65	5665	522.4	+i
32518	Metabolite - 11204	200	5.26	5263	229.2	+i
32519	Metabolite - 11205 - retired for 1-linoleoyl GPC	200	5.55	5558	520.4	+i
32520	Metabolite - 11206	200	0.59	575	138.8	+i
32578	Metabolite - 11261	200	3.69	3600	286.2	+i
32631	Metabolite - 11314	200	0.64	634	243	+i
32632	Metabolite - 11315	200	1.19	1210	130.2	+i
32644	Metabolite - 11327	200	5.16	5176	269.2	+i
32652	Metabolite - 11335	200	0.97	991	229.2	+i
32654	Metabolite - 11337	200	1	1020	160.2	+i
32671	Metabolite - 11354	200	0.76	770	146.2	+i
32738	Metabolite - 11421	200	4.54	4575	314.2	+i
32786	Metabolite - 11469	200	3.82	3874	239.1	+i
32793	Metabolite - 11476	200	4.52	4525	189.1	+i
32875	Metabolite - 11558	200	5.64	5606	420.2	+i
32971	Metabolite - 11654 - retired for isovaleryl-L-carnitine	200	2.53	2500	246.2	+i
33080	Metabolite - 11735	200	2.51	2584	207.2	+i
33081	Metabolite - 11736	200	2.58	2639	379.4	+i
33082	Metabolite - 11737	200	2.7	2747	235.2	+i
33132	Metabolite - 11787	200	1.13	1126	148.1	+i
33135	Metabolite - 11790	200	3.4	3472	823.3	+i
33138	Metabolite - 11793	200	3.57	3634	601.1	+i

33228	Metabolite - 11883	200	5.54	5524	544.3	+i
33323	Metabolite - 11977	200	3.21	3287	270.1	+i
33403	Metabolite - 12051	200	5.83	5739	456.4	+i
33408	Metabolite - 12056	200	1.12	1129	156.2	+i
33531	Metabolite - 12116	200	1.6	1640	286.1	+i
33587	Isobar: cis-9, cis-11, trans-11 eicosenoate	201	6.13	5955	309.4	-i
32747	Metabolite - 01142_201 - retired for 2-hydroxy-3-methylbutyric acid	201	1.19	1176	117.2	-i
32588	Metabolite - 01327_201	201	4.25	4242	583.2	-i
32609	Metabolite - 01345_201 - retired for epiandrosterone sulfate	201	4.9	4887	369.2	-i
32587	Metabolite - 02249_201	201	4.03	4025	267.2	-i
32550	Metabolite - 02272_201	201	1.97	1958	189	-i
32756	Metabolite - 02276_201	201	3.35	3339	199.1	-i
32553	Metabolite - 03832_201	201	2.2	2199	173.1	-i
32557	Metabolite - 06126_201	201	2.69	2684	203.1	-i
32753	Metabolite - 09789_201	201	2.62	2613	153.1	-i
32548	Metabolite - 11231	201	1.47	1471	330	-i
32561	Metabolite - 11244	201	3.78	3771	224.2	-i
32564	Metabolite - 11247	201	3.94	3932	213.1	-i
32616	Metabolite - 11299	201	4.9	4893	507.2	-i
32619	Metabolite - 11302	201	5.01	4998	397.3	-i
32625	Metabolite - 11308	201	5.15	5133	365.3	-i
32635	Metabolite - 11318	201	5.81	5699	476.3	-i
32637	Metabolite - 11320 - retired duplicate of X-12528	201	5.85	5740	593.9	-i
32648	Metabolite - 11331 - retired EDTA ions	201	0.69	686	164.2	-i
32656	Metabolite - 11339 - retired - EDTA ions	201	0.69	689	156.2	-i
32682	Metabolite - 11365 - retired for arachidonic acid	201	5.61	5527	303.3	-i
32696	Metabolite - 11379 - retired for cis-10-heptadecenoic acid	201	5.65	5566	267.3	-i
32732	Metabolite - 11415 - retired - EDTA ions	201	0.69	692	313.1	-i
32748	Metabolite - 11431	201	1.58	1575	330	-i
32754	Metabolite - 11437	201	2.89	2888	231	-i
32757	Metabolite - 11440	201	3.58	3571	246.3	-i
32760	Metabolite - 11443	201	3.92	3910	225.3	-i
32761	Metabolite - 11444	201	3.99	3983	541.2	-i
32769	Metabolite - 11452	201	4.12	4109	352.1	-i

32792	Metabolite - 11475	201	4.25	4240	383.2	-i
32795	Metabolite - 11478	201	4.3	4286	165.2	-i
32807	Metabolite - 11490	201	4.77	4762	279.8	-i
32813	Metabolite - 11496	201	5.58	5508	271.3	-i
32839	Metabolite - 11522	201	4.76	4754	313.2	-i
32848	Metabolite - 11531	201	4.86	4850	391.3	-i
32850	Metabolite - 11533	201	4.91	4904	243.2	-i
32855	Metabolite - 11538	201	4.93	4920	311.3	-i
32877	Metabolite - 11560	201	5.29	5245	295.3	-i
32910	Metabolite - 11593	201	0.79	790	189.2	-i
32970	Metabolite - 11653	201	5.82	5686	331.3	-i
33172	Metabolite - 11827	201	1.56	1575	312.1	-i
33177	Metabolite - 11832	201	1.95	1962	216.1	-i
33185	Metabolite - 11840	201	2.56	2574	135.2	-i
33190	Metabolite - 11845	201	2.87	2891	615	-i
33194	Metabolite - 11849	201	3.2	3229	266.2	-i
33198	Metabolite - 11853	201	3.59	3602	187.1	-i
33210	Metabolite - 11865	201	5.04	5037	456.2	-i
33219	Metabolite - 11874 - retired for cis-5-Dodecenoic acid	201	5.23	5199	197.3	-i
33225	Metabolite - 11880	201	5.44	5378	537.4	-i
33226	Metabolite - 11881	201	5.48	5414	380.3	-i
33227	Metabolite - 11882 - retired for cis-5,8,11,14,17-eicosapentaenoic acid	201	5.52	5445	301.3	-i
33232	Metabolite - 11887 - retired for cis-11,14-eicosadienoic acid	201	5.85	5736	307.4	-i
33237	Metabolite - 11892	201	0.71	710	367.1	-i
33380	Metabolite - 12029	201	0.68	683	329.1	-i
33388	Metabolite - 12037 - retired for cis-10-nonadecenoic acid	201	5.88	5795	295.4	-i
33389	Metabolite - 12038	201	5.82	5736	245.3	-i
33415	Metabolite - 12063	201	4.82	4822	427.2	-i
33416	Metabolite - 12064	201	1	999	101.3	-i

[00233] While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made without departing from the spirit and scope of the invention.

Claims

What is claimed is:

Claim 1. A method of predicting the glucose disposal rate in a subject, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for insulin resistance in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to glucose disposal reference levels of the one or more biomarkers in order to predict the glucose disposal rate in the subject.

Claim 2. The method of Claim 1, wherein the one or more biomarkers comprise one or more biomarkers listed in Table 4.

Claim 3. The method of Claim 1, wherein the method comprises analyzing the biological sample to determine the level of two or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 4. The method of Claim 1, wherein the method comprises analyzing the biological sample to determine the level of three or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 5. The method of Claim 1, wherein the method comprises analyzing the biological sample to determine the level of four or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 6. The method of Claim 1, wherein the method comprises analyzing the biological sample to determine the level of five or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B .

Claim 7. The method of Claim 1, wherein the method comprises analyzing the biological sample to determine the level of ten or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 8. The method of Claim 3, wherein the method comprises analyzing the biological sample to determine the level of 2-hydroxybutyrate in combination with one or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 9. The method of claim 1, wherein the method further comprises determining the subject's measurements of fasting plasma insulin, fasting plasma glucose, fasting plasma pro-insulin, fasting free fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index.

Claim 10. The method of claim 9, wherein the method comprises analyzing the subject and a biological sample from the subject using a model listed in Table 8.

Claim 11. The method of claim 1, wherein the glucose disposal rate is used to determine a level of insulin sensitivity of the subject.

Claim 12. The method of claim 11, wherein the insulin sensitivity of the subject is correlated with a glucose disposal rate.

Claim 13. A method of classifying a subject according to glucose tolerance from normal glucose tolerance (NGT), impaired fasting glucose tolerance (IFG), or impaired glucose tolerance (IGT), to type-2 diabetes, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for glucose tolerance in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to glucose tolerance reference levels of the one or more biomarkers in order to classify the subject as having NGT, IFG, IGT, or diabetic.

Claim 14. The method of claim 13, wherein the method comprises analyzing the biological sample to determine the level of two or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 15. The method of claim 13, wherein the method comprises analyzing the biological sample to determine the level of three or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 16. The method of claim 13, wherein the method comprises analyzing the biological sample to determine the level of four or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 17. The method of claim 13, wherein the method comprises analyzing the biological sample to determine the level of five or more biomarkers selected from Tables

4, 5, 6, 7, 8, 9A, and 9B.

Claim 18. The method of claim 13, wherein the method further comprises determining the subject's measurements of fasting plasma insulin, fasting plasma glucose, fasting plasma pro-insulin, fasting free fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index.

Claim 19. The method of claim 18, wherein the method comprises analyzing the subject and a biological sample from the subject using a model listed in Table 8.

Claim 20. A method of determining susceptibility of a subject to type-2 diabetes, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to diabetes-positive and/or diabetes-negative reference levels of the one or more biomarkers in order to diagnose whether the subject is susceptible to developing type-2 diabetes.

Claim 21. The method of Claim 20, wherein the method comprises analyzing the biological sample to determine the level of two or more biomarkers selected from Tables 4, 5, 6, 7, 8, 9A, and 9B.

Claim 22. The method of claim 20, wherein the method further comprises determining the subject's measurements of fasting plasma insulin, fasting plasma glucose, fasting plasma pro-insulin, fasting free fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index..

Claim 23. The method of claim 22, wherein the method comprises analyzing the subject and a biological sample from the subject using a model listed in Table 8.

Claim 24. A method of determining an insulin resistance score in a subject, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the

sample to insulin resistance reference levels of the one or more biomarkers in order to determine an insulin resistance score for the subject.

Claim 25. The method of claim 24, wherein the insulin resistance score is used to monitor the progression or regression of insulin resistance in the subject.

Claim 26. The method of claim 24, wherein the insulin resistance score is used to monitor a course of treatment in the subject.

Claim 27. A method of monitoring the progression or regression of pre-diabetes in a subject, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, 8, 9A, 9B, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to pre-diabetes progression and/or pre-diabetes-regression reference levels of the one or more biomarkers in order to monitor the progression or regression of pre-diabetes in a subject.

Claim 28. A method of monitoring the efficacy of insulin resistance treatment, the method comprising: analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, the first sample obtained from the subject at a first time point wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 4, 5, 6, 7, and 8, and combinations thereof; treating the subject for insulin resistance; analyzing a second biological sample from the subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point after treatment; comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample to assess the efficacy of the treatment for treating insulin resistance.

Claim 29. The method of claim 28, wherein the method comprises analyzing the biological samples to determine the level(s) of two or more biomarkers are selected from one or more biomarkers listed in Table 4, 5, 6, 7, and 8, and combinations thereof.

Claim 30. The method of claim 28, wherein the treatment comprises administering a therapeutic agent to the subject.

Claim 31. The method of claim 30, wherein the therapeutic agent is an insulin sensitizer

Claim 32. The method of claim 31, wherein the insulin sensitizer is a thiazolidinedione.

Claim 33. The method of claim 28, wherein the treatment comprises a lifestyle modification of the subject.

Claim 34. The method of claim 33, wherein the lifestyle modification includes a modification of the nutrition, diet, or exercise routine of the subject.

Claim 35. A method of diagnosing whether a subject has metabolic syndrome, the method comprising:

analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for metabolic syndrome in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 12 and 13, analyzing the biological sample to determine the level(s) of one or more biomarkers for glucose disposal, obesity, and/or cardiovascular disease, wherein the one or more biomarkers for glucose disposal, obesity, and/or cardiovascular disease are selected from one or more biomarkers identified in Tables 4, 5, 6, 7, 8, 9A, 9B, 14, 15, 16, 17, 21, 22, 23, 25, 26, 27, 28, and combinations thereof; and

comparing the level(s) of the one or more biomarkers in the sample to the respective disorder-positive and/or disorder-negative reference levels of the one or more biomarkers in order to diagnose whether the subject has the metabolic syndrome.

Claim 36. The method of claim 35, wherein the subject is diagnosed as having metabolic syndrome where the biological sample is positive for biomarkers for three or more conditions selected from the group consisting of metabolic syndrome, obesity, cardiovascular disease, and impaired glucose disposal.

Claim 37. The method of claim 35, wherein the subject is diagnosed as susceptible to metabolic syndrome where the biological sample is positive for biomarkers for two or more conditions selected from the group consisting of metabolic syndrome, obesity, cardiovascular disease, and impaired insulin sensitivity.

Claim 38. The method of claim 35, wherein the method further comprises determining a clinical measurement of the subject selected from the group consisting of fasting plasma insulin, fasting plasma pro-insulin, fasting plasma glucose, fasting free

fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index.

Claim 39. A method of diagnosing a cardiovascular disease in a subject, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for a cardiovascular disease in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Tables 14, 15, 16, 17, 21, 22, 23, 25, and combinations thereof; and comparing the level(s) of the one or more biomarkers in the sample to disease-positive and/or disease-negative reference levels of the one or more biomarkers in order to diagnose whether the subject has cardiomyopathy or atherosclerosis.

Claim 40. The method of Claim 39, wherein the method comprises analyzing the biological sample to determine the level of two or more biomarkers selected from Tables 14, 15, 16, 17, 21, 22, 23, and/or 25.

Claim 41. The method of Claim 39, wherein the method comprises analyzing the biological sample to determine the level of three or more biomarkers selected from Tables 14, 15, 16, 17, 21, 22, 23, and/or 25.

Claim 42. The method of Claim 39, wherein the method comprises analyzing the biological sample to determine the level of four or more biomarkers selected from Tables 14, 15, 16, 17, 21, 22, 23, and/or 25.

Claim 43. The method of Claim 39, wherein the method comprises analyzing the biological sample to determine the level of five or more biomarkers selected from Tables 14, 15, 16, 17, 21, 22, 23, and/or 25.

Claim 44. The method of Claim 39, wherein the method comprises analyzing the biological sample to determine the level of ten or more biomarkers selected from Tables 14, 15, 16, 17, 21, 22, 23, and/or 25.

Claim 45. The method of Claim 39, wherein the cardiovascular disease is cardiomyopathy.

Claim 46. The method of claim 45, wherein the one or more biomarkers comprise one or more biomarkers listed in Tables 21, 22, 23 and/or 25.

Claim 47. The method of Claim 39, wherein the cardiovascular disease is atherosclerosis.

Claim 48. The method of Claim 47, wherein the one or more biomarkers comprise one or more biomarkers listed in Tables 14, 15, 16, and/or 17.

Claim 49. The method of claim 48, wherein the biological sample is analyzed for each of the biomarkers 3-methylhistidine, p-cresol sulfate, mannose, glucose, and gluconate.

Claim 50. A method for determining whether a subject is predisposed to becoming obese, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for obesity in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Table 26; and comparing the level(s) of the one or more biomarkers in the sample to obesity-positive and/or obesity-negative and/or lean-positive and/or lean-negative reference levels of the one or more biomarkers in order to determine whether the subject is susceptible to obesity.

Claim 51. A method for determining whether a therapeutic agent is capable of inducing weight gain in a subject, the method comprising: analyzing a biological sample from a subject receiving a therapeutic agent to determine the level(s) of one or more biomarkers for obesity in the sample, wherein the one or more biomarkers are selected from one or more biomarkers listed in Table 26; and comparing the level(s) of the one or more biomarkers in the sample to obesity-positive and/or obesity-negative and/or lean-positive and/or lean-negative reference levels of the one or more biomarkers in order to determine whether the subject is susceptible to gaining weight.

Claim 52. A method for predicting a subject's response to a course of treatment for pre-diabetes or diabetes, the method comprising: analyzing a biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, wherein the one or more biomarkers are selected from one or more biomarkers listed in Table 27; comparing the level(s) of one or more biomarkers in the sample to treatment-positive and/or treatment-negative reference levels of the one or more biomarkers to predict whether the subject is likely to respond to a course of treatment.

Claim 53. The method of claim 52, wherein the method comprises analyzing the biological samples to determine the level(s) of two or more biomarkers are selected from one or more biomarkers listed in Table 27, and combinations thereof.

Claim 54. The method of claim 52, wherein the course of treatment is a therapeutic agent.

Claim 55. The method of claim 54, wherein the therapeutic agent is an insulin sensitizer

Claim 56. The method of claim 55, wherein the therapeutic agent is a thiazolidinedione.

Claim 57. The method of claim 52, wherein the method further comprises determining the subject's measurements of fasting plasma insulin, fasting plasma pro-insulin, fasting plasma glucose, fasting free fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index.

Claim 58. A method for monitoring a subject's response to a treatment for pre-diabetes or diabetes, the method comprising: analyzing a first biological sample from a subject to determine the level(s) of one or more biomarkers for pre-diabetes, the first sample obtained from the subject at a first time point wherein the one or more biomarkers are selected from one or more biomarkers listed in Table 28; administering the composition to the subject; analyzing a second biological sample from the subject to determine the level(s) of the one or more biomarkers, the second sample obtained from the subject at a second time point after administration of the composition; comparing the level(s) of one or more biomarkers in the first sample to the level(s) of the one or more biomarkers in the second sample to assess the efficacy of the composition for treating pre-diabetes or diabetes.

Claim 59. The method of claim 58, wherein the composition is an insulin sensitizer

Claim 60. The method of claim 59, wherein the insulin sensitizer is a thiazolidinedione.

Claim 61. The method of claim 58, wherein the method further comprises determining the subject's measurements of fasting plasma insulin, fasting plasma pro-insulin, fasting plasma glucose, fasting free fatty acids, HDL-cholesterol, LDL-cholesterol, C-peptide, adiponectin, peptide YY, hemoglobin A1C, waist circumference, body weight, or body mass index.