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(54) **MULTIPLE-MARKER RISK PARAMETERS PREDICTIVE OF CONVERSION TO DIABETES**

MULTIMARKER-RISIKOPARAMETER ALS PRÄDIKTOREN DER KONVERSION IN DIABETES

PARAMÈTRES DE RISQUE À MARQUEUR MULTIPLE PRÉDICTIFS DE CONVERSION AU DIABÈTE

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(73) Proprietor: **Liposcience, Inc.**
Raleigh, NC 27616 (US)

(72) Inventors:
- **OTVOS, James D.**
Cary, North Carolina 27511 (US)
- **SHALAUROVA, Irina Y.**
Cary, North Carolina 27519 (US)

(74) Representative: **Yeadon IP Limited**
Nexus
Discovery Way
Leeds LS2 3AA (GB)

(56) References cited:
WO-A1-2013/185014 **WO-A1-2013/185014**
US-A1- 2010 100 334

- **IRINA SHALAUROVA ET AL: "Lipoprotein Insulin Resistance Index: A Lipoprotein Particle-Derived Measure of Insulin Resistance", METABOLIC SYNDROME AND RELATED DISORDERS, vol. 12, no. 8, 1 October 2014 (2014-10-01), pages 422-429, XP055355450, US ISSN: 1540-4196, DOI: 10.1089/met.2014.0050**
- **BARCLAY, ALAN W. ET AL.: 'Glycemic index, glycemic load, and chronic disease risk-a meta-analysis of observational studies' THE AMERICAN JOURNAL OF CLINICAL NUTRITION vol. 87, no. 3, 01 March 2008, pages 627 - 637, XP055347498**
- **LU , SHOU-EN ET AL.: 'Evaluation of risk equations for prediction of short- term coronary heart disease events in patients with long-standing type 2 diabetes: the Translating Research into Action for Diabetes (TRIAD) study' BMC ENDOCRINE DISORDERS vol. 12, no. 1, 09 July 2012, pages 1 - 10, XP021115730 DOI: 10.1186/1472-6823-12-12**
- **O'CONNELL, THOMAS M.: 'The complex role of branched chain amino acids in diabetes and cancer' METABOLITES vol. 3, no. 4, 01 January 2013, pages 931 - 945, XP055347502 DOI: 10.3390/METABO3040931**

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Description

Reservation of Copyright

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Field of the Invention

[0002] The present invention relates generally to analysis of *in vitro* biosamples.

Background of the Invention

[0003] Type 2 diabetes mellitus (T2DM or "diabetes") is one of the most costly and burdensome chronic diseases in the U.S. and other countries. The defining feature of T2DM is hyperglycemia, a reflection of impaired carbohydrate (glucose) utilization resulting from a defective or deficient insulin secretory response. T2DM is a late manifestation of metabolic derangements that begin many years earlier. Its cause is believed to be a progressive increase in insulin resistance coupled with deteriorating β-cell function. So long as the pancreatic β-cells are able to secrete enough insulin to compensate for the progressive resistance of target tissues to insulin's hypoglycemic effects, the patient is able to maintain normal fasting glucose levels. Hyperglycemia and the transition to T2DM occur as a consequence of progressive β-cell dysfunction which leads to failure to maintain hypersecretion of insulin in the face of increasing insulin resistance.

[0004] Type 2 diabetes has been traditionally diagnosed by the detection of elevated levels of glucose (sugar) in the blood (hyperglycemia). While hyperglycemia defines diabetes, it is a very late stage development in the chain of events that lead from insulin resistance to full-blown diabetes. Accordingly, it would be desirable to have a way of identifying whether or not a subject is at risk for developing Type 2 diabetes (*i.e.,* is predisposed to the condition) prior to the development of the classic symptoms, such as hyperglycemia. Earlier detection of indicators of the disease (e.g., detection before glucose levels are elevated enough to be considered hyperglycemia) may lead to more effective treatment of the disease, if not actual prevention of the onset of the disease.

[0005] The most direct and accurate methods for assessing insulin resistance are laborious and time-consuming, and thus impractical for clinical application. The "gold standard" among these research methods is the hyperinsulinemic euglycemic clamp, which quantifies the maximal glucose disposal rate (GDR, inversely proportional to insulin resistance) during the clamp. Another arduous research method which is somewhat less reproducible (CV 14-30%) is the frequently sampled intravenous glucose tolerance test (IVGTT) with minimal model analysis, which measures insulin sensitivity (S_i), the inverse of insulin resistance.

[0006] Risk of progression to Type 2 diabetes is currently assessed primarily by fasting glucose, with concentrations 100-125 mg/dL defining a high-risk "pre-diabetes" condition and for which T2DM is currently defined in patients having fasting plasma glucose levels at 126 mg/dL and above. However, the actual risk of individual patients with pre-diabetes (those at greatest risk of developing T2DM in the near future) varies widely.

[0007] NMR spectroscopy has been used to concurrently measure low density lipoproteins (LDL), high density lipoproteins (HDL), and very low density lipoproteins (VLDL), as LDL, HDL and VLDL particle subclasses from *in vitro* blood plasma or serum samples. *See,* U.S. Patent Nos. 4,933,844 and 6,617,167. U.S. Patent No. 6,518,069 to Otvos et al. describes NMR derived measurements of glucose and/or certain lipoprotein values to assess a patient's risk of developing T2DM.

[0008] Generally stated, to evaluate the lipoproteins in a blood plasma and/or serum sample, the amplitudes of a plurality of NMR spectroscopy derived signals within a chemical shift region of NMR spectra are derived by deconvolution of the composite methyl signal envelope to yield subclass concentrations. The subclasses are represented by many (typically over 60) discrete contributing subclass signals associated with NMR frequency and lipoprotein diameter. The NMR evaluations can interrogate the NMR signals to produce concentrations of different subpopulations, typically seventy-three discrete subpopulations, 27 for VLDL, 20 for LDL and 26 for HDL. These sub-populations can be further characterized as associated with a particular size range within the VLDL, LDL or HDL subclasses.

[0009] An advanced lipoprotein test panel, such as the LIPOPROFILE® lipoprotein test, available from LipoScience, Raleigh, N.C., has typically included a total high density lipoprotein particle (HDL-P) measurement (e.g., HDL-P number) that sums the concentration of all the HDL subclasses and a total low density lipoprotein particle (LDL-P) measurement that sums the concentration of all the LDL subclasses (e.g., LDL-P number). The LDL-P and HDL-P numbers represent the concentration of those respective particles in concentration units such as nmol/L. LipoScience has also developed a lipoprotein-based insulin resistance and sensitivity index (the "LP-IR™" index) as described in U.S. Patent No.

8,386,187.

[0010] **Figure 1A** illustrates a timeline over which insulin resistance and insulin production change before onset of T2DM, with β-cell dysfunction occurring during the progression. Glucose may remain relatively stable before rapidly increasing during progression to T2DM. *See, e.g.,* Mason et al., Diabetes 2007; 56: 2054-6. **Figure 1B** illustrates various glucose ranges and a continuum of T2DM progression during which diabetic complications can occur. Many with glucose in the 100-110 mg/dL range do not progress to T2DM and others with "normal" glucose in the range of 90-99 mg/dL, for example, may develop diabetes in less than five years. Currently the risk of diabetes progression is primarily evaluated by glucose measures that are the response to, not the cause of, worsening glucose metabolism. There is an unmet diagnostic need for tests that can better distinguish between those that will progress to diabetes from those that will not. *See,* AACE Prediabetes Consensus Statement, Endocr Pract. 2008; 14 (No. 7) 941.

[0011] There remains a need for evaluations that can predict or assess a person's risk of developing type 2 diabetes before the onset of the disease and/or to identify those individuals at risk of progression early so that interventions may be used to delay progression and/or treat cardiovascular risk.

Summary

[0012] The invention is defined in claims 1 and 12. Embodiments of the invention provide multimarkers of (i) insulin resistance ("IR"), (e.g, eLP-IR) and (ii) short-term diabetes risk factors (SDRF) of fasting plasma biosamples that can assess diabetes risk at any level of glycemia.

[0013] Embodiments of the invention provide risk assessments of a subject's risk of developing type-2 diabetes in the future using multi-parameter (multi-variate) risk progression models, one model for SDRF associated with short term risk ("STR") (e.g., less than 3 years, such as within 6 months, 1 year, 2 year or 3 years, post test) and another for IR which can be an important risk parameter for both STR and longer term risk ("LTR") (e.g., greater than 3 years, such as 3.5 years, 4 years, 5 years or between 5-10 years, post test).

[0014] The SDRF can be provided as a score. The SDRF score is positively associated with short term risk and therefore is thought to be inversely related to beta cell function or positively related to pancreatic beta cell dysfunction.

[0015] The IR score can be identified using an "eLP-IR" (extended lipoprotein insulin resistance) score.

[0016] The STR model (assessed with logistic regression). The IR model can be assessed with linear regression models for either or both HOMA-IR or Si (insulin sensitivity, as assessed by frequently sampled intravenous glucose tolerance testing) based on at least one study population.

[0017] Embodiments of the invention may use the multi-parameter diabetes risk parameters as inputs to a diabetes risk model to generate a diabetes risk index (DRI) score associated with risk of conversion within a defined timeline, typically five years.

[0018] Coefficients for the risk models can be defined by logistic regression for respective short term and longer term diabetes conversion (per the IR model) to generate an equation that can combine the various marker inputs.

[0019] The SDRF parameter (for STR) and the IR parameter can be provided as a numerical score or value in a defined range, lower numbers for lower risk and higher values for higher risk. In some embodiments, the SDRF and IR scores can be combined to provide a diabetes risk index ("DRI") score.

[0020] Embodiments of the invention may provide a logistic regression model with components from both the SDRF and IR models for a DRI score or a "diabetes risk index" that typically is associated with a timeline of between 3-7 years, e.g., about 5 years, post-test.

[0021] Embodiments of the invention can generate an STR evaluation that can be used to identify at-risk patients that may benefit from therapies for improving or stabilizing beta-cell function and/or improve a patient's ability to produce insulin. It is contemplated that a nonlimiting example of such a therapy can be reconstituted HDL infusion therapy that has been used for post-myocardial infarction patients.

[0022] Embodiments of the invention can provide STR scores for drug therapy, drug discovery and/or clinical trials. The STR score can be used as a marker to identify a dysfunction and/or a change in beta-cell function.

[0023] The STR score can be a relative score or an absolute score relative to a defined population. The STR score can be used with a baseline STR score and a subsequent STR score for a subject where a change reflects a change from an administered therapy or drug discovery program.

[0024] The STR, IR and/or DRI scores can be generated before, during and/or after administration of a drug therapy to identify a change in respective scores for a subject and therefore identify beneficial or negative change from the drug therapy.

[0025] The SDRF and/or STR score can be used for evaluating therapies or drugs that may improve or stabilize beta-cell function and/or the ability to produce insulin or for evaluating undesired side-effects of drugs.

[0026] The risk assessments can generate respective STR, IR, and DRI scores that stratify risk beyond glucose measurements alone and may be decoupled from glucose measurements.

[0027] The DRI score, where used, may be based on risk prediction models of about a 5 year risk of conversion using

some or all components from both the SDRF and IR risk prediction models, which may be weighted using different defined weighting factors to generate the DRI score. The risk assessments can consider glucose measurements. Where used, a glucose measurement can help establish a timeline of conversion to type 2 diabetes and/or be used in evaluation of risk. The diabetes risk index scores can be used without glucose information and may reflect risk over both a short term and a longer term associated with underlying metabolic issues.

[0028] The multi-variate models can be used for assessing patients for or during clinical trials, during a therapy or therapies, for drug development, for drug therapy selection or indication for a subject, and/or to identify or monitor anti-obesity drugs or other drug therapy candidates.

[0029] The short term multi-variate model can include NMR measurements of GlycA, a lipoprotein component (HMP) associated with concentration of a defined subpopulation of high density lipoprotein (HDL) particles, and an interaction parameter of the measurement of GlycA multiplied by HMP (the concentration of a defined subpopulation of high density lipoprotein (HDL) particles), HMPxGlycA.

[0030] While GlycA may be a preferred inflammation marker, other inflammation markers may be used including, for example, fibrinogen, haptoglobin, alpha-1-acid glycoprotein, CRP (C-reactive protein), hs)CRP (high sensitivity CRP), or IL-L (interleukin-6).

[0031] The HDL subpopulation can include only medium HDL particle subclasses (HMP). Medium HDL-P refers to a sub-population of HDL particles that excludes small and large HDL particle subclasses and the exact size range may vary between measurement methodologies and study populations which maximize risk of diabetes using a risk model (typically a logistic regression model of at least one defined study population), either or both in the short term or a risk model based on IR for a longer term.

[0032] By way of example only, the medium HDL-P may include only HDL particles with diameters between about 8.3 nm (average) to an upper value of 9.4 nm (average), or 10.0 nm (average) or 10.2 nm (average).

[0033] The SDRF and/or STR risk model can include components that are markers of impaired insulin secretion and/or pancreatic beta-cell dysfunction.

[0034] The DRI risk model can include components that are markers of insulin resistance as well as the SDRF markers or the SDRF score.

[0035] The IR multi-variate model can include at least one defined lipoprotein component and at least one defined branched chain amino acid. Optionally, the model may include at least one inflammatory biomarker. The longer term (IR) multi-variate model can include Valine, and a plurality of lipoprotein components (e.g., subclasses) derived from the same NMR spectrum. The plurality of lipoprotein components can include LP-IR and VMP (the concentration of a defined subpopulation of very low density lipoprotein particles, the medium VLDL subclass particle number or "medium VLDL-P").

[0036] The term "medium VLDL particles" or "VMP" refers to a concentration of particles with diameters/sizes between 35-60 nm (average).

[0037] The DRI risk model can include components from the SDRF and IR models, typically three from each including HMP, GlycA, HMPxGlycA from the STR model and LP-IR, Valine and med-VLDL-P from the IR model.

[0038] Embodiments of the invention include methods, circuits, NMR spectrometers or NMR analyzers, and processors that evaluate a future risk of developing diabetes and/or risk stratification for those having normal glucose or slightly elevated glucose by evaluating NMR spectra of an *in vitro* blood plasma or serum patient sample using defined short term and longer term multi-component risk progression models.

[0039] In some embodiments, the STR and IR scores can be weighted to generate a DRI score in a defined numerical range. In some embodiments, the IR score can be weighted to account for a greater contribution that IR makes to risk over a 5-year (or other longer term conversion time) relative to SDRF.

[0040] Embodiments of the invention are directed to methods of evaluating a subject's risk of developing type 2 diabetes and/or having pancreatic beta cell impairment and/or dysfunction. The methods include: programmatically calculating a short term diabetes risk factor (SDRF) score of a subject using a defined mathematical model of risk of developing type 2 diabetes. The defined mathematical model includes a concentration measurement of a defined high density lipoprotein particle (HDL-P) subpopulation, a concentration measurement of at least one inflammatory marker and a concentration measurement of at least one interaction parameter as components of the model which are mathematically combined with respective defined coefficients to generate the calculated SDRF score. The subject is at risk of converting to type 2 diabetes mellitus within 3 years and/or is at risk of beta cell dysfunction when the SDRF score is at a third tertile value or greater value of a population norm.

[0041] The defined HDL-P subpopulation is medium HDL-P.

[0042] The inflammatory marker can be GlycA. The interaction parameter can be GlycA multiplied by the concentration of medium HDL-P. The subject is at risk of beta cell dysfunction when medium HDL-P and Glyc A values are in a third tertile values of the population norm.

[0043] The method can include programmatically calculating an insulin resistance (IR) score of the subject using a defined mathematical model of insulin resistance.

[0044] The defined mathematical model of insulin resistance for the IR score can include a plurality of components including a concentration measurement of a defined HDL-P subpopulation, which may be the concentration measurement of the defined HDL-P subpopulation used to calculate the SDRF score, the measurement of the inflammatory marker, a measurement of a defined subpopulation of VLDL-P (very low density lipoprotein /chylomicron particle subclasses), an IR index with a range of between 0-100, the range representing from low to high, insulin sensitivity to insulin resistance, and a measurement of a branched chain amino acid (BCAA), obtained from the at least one *in vitro* biosample of the subject.

[0045] The components of the defined mathematical model of IR can be mathematically combined to generate the IR score.

[0046] The measurement of the defined sub-population of VLDL-P can be a concentration of medium VLDL-P, and wherein the BCAA is Valine.

[0047] The method can include programmatically calculating a diabetes risk score by combining the SDRF score and the IR score.

[0048] The coefficients of the SDRF and IR scores can be derived from a logistic regression model that includes SDRF and IR to predict actual 5 year conversion to diabetes using at least one defined population study to thereby generate a DRI score with a risk of conversion within 5 years irrespective of a glucose value of the subject.

[0049] The method can include evaluating a measured glucose and/or HbAlc value of the subject and electronically providing a report with a 5 year risk of conversion to Type 2 diabetes risk estimate based on the glucose measurement and the DRI score.

[0050] The SDRF score can be calculated using the following equation: SDRF score = - (A) (HDL-P_{MED}) - (B) (GlycA) + (C) (GlycA x HDL-P_{MED}), where A, B and C can be defined beta coefficients from a logistic regression model for short term conversion to diabetes as the defined mathematical model of risk of developing type 2 diabetes. GlycA can be the inflammatory marker, HDL-P_{MED} is a medium size HDL-P subpopulation, and GlycA x HDL-P_{MED} can be the interaction parameter.

[0051] The IR score can be an eLP-IR score and is calculated using the following equation:

$$\text{eLP-IR} = (A)\ (\text{LP-IR}) + (B)(\text{Valine}) - (C)\ (\text{VLDL-P}_{MED}) - (D)(\text{HDL-P}_{MED}) + (E)(\text{GlycA}).$$

A, B, C, D and E can be defined beta coefficients from a linear regression model for insulin resistance. GlycA can be the inflammatory marker, HDL-P_{MED} can be the concentration of a medium HDL-P subpopulation, VLDL-P_{MED} can be a concentration of a medium VLDL-P subpopulation, valine can be a branched chain amino acid, and LP-IR can be a lipoprotein insulin resistance index calculated using six defined lipoprotein subclasses and has a numerical value in a range of 0-100 representing insulin sensitive to insulin resistance.

[0052] The method can also include at least one of: evaluating a drug therapy, evaluating a clinical trial, or evaluating candidates for drug discovery, using the SDRF score.

[0053] The method can include calculating a plurality of the SDRF scores over time from respective biosamples to thereby evaluate a change in SDRF score to identify a change in β-cell dysfunction.

[0054] The raw scores associated with the SDRF score can be between -6.4 and -1.6, wherein -4.1 can be associated with about a 25th percentile and >-3.8 can be associated with about a 75th percentile of the study population. Scores ≥-3.8 values can indicate an increased risk of beta cell dysfunction and/or early conversion to type 2 diabetes independent of glycemic value that can stratify risk of conversion to type 2 diabetes for subjects having a common glycemic measurement with different SDRF scores.

[0055] The SDRF score can be provided in a report in a defined numerical score range, with scores associated with a fourth quartile (4Q), fifth quintile (5Q) or 10th decile of a population norm reflecting an increased risk of developing type 2 diabetes within 2 years and/or beta cell dysfunction and/or impairment relative to lower scores.

[0056] The HDL-P subpopulation can include only medium HDL particle subclasses with diameters between 8.3 nm to one of : 9.4 nm, 10.0 nm or 10.2 nm.

[0057] The method can include generating a DRI score using the following equation: DRI score = X(IR score) + Y(SDRF). X and Y can be coefficients defined by a logistic regression model for 5-year diabetes conversion in people with glucose <110 mg/dL using a defined study population, and wherein the DRI score is mathematically altered into a DRI score range using a plurality of equal subparts over a range of possible DRI raw scores.

[0058] The raw DRI scores may be between about -3.0 to about 1.8.

[0059] Embodiments of the invention can be directed to methods of identifying at-risk patients that may benefit from therapies for improving or stabilizing beta-cell function and/or improve a patient's ability to produce insulin. The methods can include electronically generating a short term risk score by combining measurements of defined lipoprotein and metabolite components of a biosample of a subject, wherein the components include a high density lipoprotein particle (HDL-P) subpopulation and an interaction parameter using the HDL-P subpopulation and an inflammatory marker.

[0060] Other embodiments are directed to methods of identifying subjects that are likely to benefit from a drug therapy such as reconstituted HDL infusion for improving pancreatic beta cell function and/or to inhibit Type 2 diabetes mellitus (T2DM). The methods can include

generating a defined short term diabetes risk factor (SDRF) score using measurements of defined lipoprotein and metabolite components of a biosample of a subject. The components include a high density lipoprotein particle (HDL-P) subpopulation and an interaction parameter using the HDL-P subpopulation and an inflammatory marker; and identifying subjects that have an increased SDRF score relative to a defined population norm which indicates that the subject is likely to benefit from therapy to improve pancreatic beta cell function and/or inhibit T2DM.

[0061] The methods can be carried out using at least one processor.

[0062] All or some of the measurements of the biosample for the SDRF score can all be NMR derived measurements.

[0063] Other embodiments are directed to methods of evaluating a patient's risk of conversion to type 2 diabetes by:

(a) programmatically calculating a SDRF score using the following equation: SDRF score = - (A) (HDL-P_{MED}) - (B) (GlycA) + (C) (GlycA x HDL-P_{MED}),

wherein A, B and C are defined beta coefficients from a logistic regression model for short term conversion to diabetes as the defined mathematical model of risk of developing type 2 diabetes, and wherein GlycA is an inflammatory marker, HDL-P_{MED} is a medium size HDL-P subpopulation, and GlycA x HDL-P_{MED} is an interaction parameter;

(b) programmatically calculating an eLP-IR score and using the following equation:

$$\text{eLP-IR} = (A)\ (\text{LP-IR}) + (B)(\text{Valine}) - (C)\ (\text{VLDL-P}_{MED}) - (D)(\text{HDL-P}_{MED}) + (E)(\text{GlycA}),$$

wherein A, B, C, D and E are defined beta coefficients from a linear regression model for insulin resistance, wherein GlycA is the inflammatory marker, HDL-P_{MED} is the concentration of a medium HDL-P subpopulation, VLDL-P_{MED} is a concentration of a medium VLDL-P subpopulation, valine is a branched chain amino acid, and LP-IR is a lipoprotein insulin resistance index calculated using six defined lipoprotein subclasses and has a numerical value in a range of 0-100 representing insulin sensitive to insulin resistance, and

(c) programmatically generating a DRI raw score using the following equation: DRI raw score = X(eLP-IR) + Y(SDRF), wherein X and Y are coefficients defined by a logistic regression model for 5-year diabetes conversion in people with glucose <110 mg/dL using a defined study population, and wherein the DRI raw score is mathematically altered into a range of between 0-10 or 1-10 using a plurality of equal subparts over a range of possible DRI raw scores.

[0064] Further features, advantages and details of the present invention will be appreciated by those of ordinary skill in the art from a reading of the figures and the detailed description of the preferred embodiments that follow, such description being merely illustrative of the present invention. Features described with respect with one embodiment can be incorporated with other embodiments although not specifically discussed therewith. That is, it is noted that aspects of the invention described with respect to one embodiment, may be incorporated in a different embodiment although not specifically described relative thereto. That is, all embodiments and/or features of any embodiment can be combined in any way and/or combination. Applicant reserves the right to change any originally filed claim or file any new claim accordingly, including the right to be able to amend any originally filed claim to depend from and/or incorporate any feature of any other claim although not originally claimed in that manner. The foregoing and other aspects of the present invention are explained in detail in the specification set forth below.

[0065] As will be appreciated by those of skill in the art in light of the present disclosure, embodiments of the present invention may include methods, systems, apparatus and/or computer program products or combinations thereof.

Brief Description of the Figures

[0066] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and payment of the necessary fee.

Figure 1A is a chart illustrating pathophysiology of Type 2 diabetes progression.

Figure 1B is a chart illustrating a continuum of Type 2 diabetes progression with examples of worsening diabetic complications.

Figure 2A is a chart illustrating diabetes risk prediction based on two separate multi-parameter risk models, one for short term risk associated with beta cell dysfunction and one for IR according to embodiments of the present invention.

Figure 2B is a chart illustrating diabetes risk prediction based on two separate multi-parameter risk models with

exemplary particular components for the inflammatory marker and BCAA (branched chain amino acid), one for short term risk and one for insulin resistance according to embodiments of the present invention.

Figures 3A and **3B** illustrate subpopulations of lipoproteins with exemplary size ranges according to embodiments of the present invention.

Figure 4 is a chart of exemplary HDL subpopulation groupings for diabetes risk assessment based on associations (positive and negative) with incident diabetes according to embodiments of the present invention.

Figure 5 is a graph showing risk associations for different sub-populations of HDL per the size groupings in **Figure 4** according to embodiments of the present invention.

Figure 6A is a graph of diabetes conversion rate versus glucose category (low risk, intermediate risk, high risk) based on MESA 5-year T2DM conversion (n=359/4211) illustrating an unmet need for stratifying or better identifying patients at increased risk of progression to T2DM for intermediate risk glucose patients according to embodiments of the present invention.

Figure 6B is a graph of diabetes conversion rate versus glucose category (low risk, intermediate risk, high risk) based on IRAS 5-year T2DM conversion (n=134/978) illustrating an unmet need for stratifying or better identifying patients at increased risk of progression to T2DM for intermediate risk glucose patients according to embodiments of the present invention.

Figure 7 is a table of the characteristics of the MESA study population (n=3450).

Figure 8 is an NMR spectrum illustrating a GlycA peak region (over an enlarged view of the region) according to embodiments of the present invention.

Figure 9 is a graph of predicted short term diabetes conversion rate by GlycA level for three defined levels (high, intermediate and low) of medium HDL-P ($HDL\text{-}P_{MED}$) illustrating high levels of medium HDL-P turning from "good" to "bad" at about an 80^{th} percentile level of GlycA (relative to a population norm) according to embodiments of the present invention.

Figure 10 is a block diagram similar to **Figure 2,** illustrating exemplary coefficients that may be used with the noted respective components to generate eLP-IR and SDRF scores, which may be used alone or combined to form a DRI score according to embodiments of the present invention.

Figure 11A is a graph of predicted diabetes conversion rates (%) versus glucose category in mg/dL units by DRI score (n=1-10) in Multi-Ethnic Study of Atherosclerosis (MESA) according to embodiments of the present invention.

Figure 11B is a graph of predicted diabetes conversion rates (%) versus glucose category in mg/dL units by DRI score (n=1-10) in IRAS (Insulin Resistance Atherosclerosis Study) according to embodiments of the present invention.

Figure 12 is a chart of parameters contributing to the eLP-IR multimarker parameter of insulin resistance according to embodiments of the present invention.

Figure 13 is a chart of parameters contributing to the SDRF multimarker parameter of short term risk according to embodiments of the present invention.

Figure 14 is a chart illustrating the conversion to diabetes during 5.2 year follow-up in IRAS (n=134/976) which validates the model developed using MESA according to embodiments of the present invention.

Figure 15 is a chart illustrating the actual rates (in percent) of 5-year conversion to diabetes within 9 subgroups of IRAS participants (all with fasting glucose less than or equal to 110 mg/dL) subdivided according to their levels (low, intermediate or high) of GlycA and medium HDL-P ($HDL\text{-}P_{MED}$) according to embodiments of the present invention. Among the noted 850 participants, 88 converted to diabetes.

Figure 16 is an exemplary graph of fasting glucose levels (mg/dL) over time for hypothetical patients with high and low levels of insulin resistance and SDRF to illustrate that SDRF and IR (eLP-IR, for example) can be used to predict whether the glucose value will be stable over time or have a trajectory that leads to diabetes in the future, e.g., either in the short term or longer term according to embodiments of the present invention. The color coding distinguishes those three patients with stable glucose levels over time (green) from those who are likely to convert to diabetes (red).

Figure 17 is an exemplary report which provides a measure of short term risk, e.g., a SDRF score, which may be monitored for change to assess β-cell function/dysfunction over time.

Figure 18 is a schematic illustration of an exemplary patient report that can provide one or more of a DRI score, IR score (associated with insulin resistance) and STR score (associated with β-cell function) according to embodiments of the present invention.

Figure 19 is an exemplary graph that can be used to evaluate change in one or more of a DRI score, an IR score or an STR scores over time, which may be based or correlated to doses or types of therapies according to embodiments of the present invention.

Figures 20A-20C illustrate exemplary patient reports with DRI scores associated with risk categories to predict a conversion to T2DM within a defined timeline such as 5 years according to embodiments of the present invention.

Figure 21 is a schematic illustration of a system for analyzing a patient's risk using a STR, IR and/or DRI risk index module and/or circuit using according to embodiments of the present invention.

Figure 22 is a schematic illustration of a NMR spectroscopy apparatus according to embodiments of the present invention.
Figure 23 is a schematic diagram of a data processing system according to embodiments of the present invention.
Figure 24 is a flow chart of exemplary operations that can be used to assess a risk of developing T2DM in the future and/or having prediabetes, according to embodiments of the present invention.

[0067] The foregoing and other objects and aspects of the present invention are explained in detail in the specification set forth below.

Detailed Description of Embodiments of the Invention

[0068] The present invention now is described more fully hereinafter with reference to the accompanying drawings, in which embodiments of the invention are shown. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.
[0069] Like numbers refer to like elements throughout. In the figures, the thickness of certain lines, layers, components, elements or features may be exaggerated for clarity. The term "Figure" is used interchangeably with the abbreviated versions "FIG." and "Fig." in the specification and figures.
[0070] Broken lines illustrate optional features or operations unless specified otherwise.
[0071] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. It will be further understood that the terms "comprises" and/or "comprising," when used in this specification, specify the presence of stated features, integers, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, integers, steps, operations, elements, components, and/or groups thereof. As used herein, the term "and/or" includes any and all combinations of one or more of the associated listed items. As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y." As used herein, phrases such as "from about X to Y" mean "from about X to about Y."
[0072] Exemplary descriptions of components of risk progression models for diabetes risk indexes or scores are described in U.S. Patent Application Serial Number 13/830,784, filed March 14, 2013, and PCT/US2013/044679, filed June 7, 2013 (WO 2013/185014).
[0073] Unless otherwise defined, all terms (including technical and scientific terms) used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. It will be further understood that terms, such as those defined in commonly used dictionaries, should be interpreted as having a meaning that is consistent with their meaning in the context of the specification and relevant art and should not be interpreted in an idealized or overly formal sense unless expressly so defined herein. Well-known functions or constructions may not be described in detail for brevity and/or clarity.
[0074] The term "about" refers to +/- 10% (mean or average) of a specified value or number.
[0075] The term "prediabetes" refers to a risk state for a patient or subject rather than a disease state. Thus, the term "prediabetes" refers to someone that has not been diagnosed with type 2 diabetes and, as currently defined by the American Diabetes Association, is associated with individuals that have a fasting plasma glucose level that is between 100 and 125 mg/dL, an oral glucose tolerance test level that is between 140-199 (mg/dL) or an A1C percent that is between 5.7 to 6.4 as represented in **Table 1** below (the greater the level, the higher the risk of type 2 diabetes for each type of test).

Table 1: Blood Test Levels for Diabetes and Prediabetes

	A1C (percent)	Fasting Plasma Glucose (mg/dL)	Oral Glucose Tolerance Test (mg/dL)
Diabetes	**6.5 or above**	**126 or above**	**200 or above**
Prediabetes	**5.7 to 6.4**	**100 to 125**	**140 to 199**

(continued)

	A1C (percent)	Fasting Plasma Glucose (mg/dL)	Oral Glucose Tolerance Test (mg/dL)
Normal	**About 5**	**99 or below**	**139 or below**
Definitions: mg = milligram, dL = deciliter For all three tests, within the prediabetes range, the higher the test result, the greater the risk of diabetes. *See,* American Diabetes Association. Standards of medical care in diabetes-2012. Diabetes Care. 2012:35 (Supp 1):S12, table 2.			

[0076] Embodiments of the invention may be particularly suitable to stratify risk for patients having the same or similar fasting glucose levels. Generally stated, it is contemplated that STR and IR scores, alone or combined into a DRI score, can be used to stratify risk for developing type 2 diabetes in the future alone or with FPG or other measure of glucose such as A1C (a non-fasting sample using hemoglobin A1C) or oral glucose tolerance measurements. One or more of a STR, IR or DRI diabetes risk score can be used to stratify type 2 diabetes risk for patients having the same glucose level, but different underlying metabolic situations.

[0077] The connection of SDRF with beta cell dysfunction is currently theoretical as no direct evidence that SDRF is actually associated with an objective measure of beta cell function (of which there aren't many and they aren't very good) has been established as of the filing date of this patent application. However, since SDRF contributes only to short but not long-term risk and is independent of IR, a connection to beta cell dysfunction is strongly implied, if currently only an inference.

[0078] Embodiments of the invention can provide a "cumulative" diabetes risk based on IR status and beta cell function, within a relatively short timeframe, typically 5 years. This risk is contributed to by insulin resistance (a necessary ingredient of diabetes risk no matter what the timeframe) and also by beta-cell dysfunction which is typically a late manifestation (influencing only relatively short-term conversion, typically within 2-3 years). So to assess risk of conversion to T2DM within 5 years, an assessment of both IR (e.g., eLP-IR) and beta-cell dysfunction (SDRF) can be used. The relative importance of the beta-cell part of the overall risk is greater if the timeframe of interest is shorter rather than longer, maybe accounting for ~50% of the risk of 3-year conversion, ~30% of the risk of 5-year conversion, and <10% of the (cumulative) risk of 10-year conversion. Thus, embodiments of the invention can provide a new multimarker of 5-yr diabetes risk (a DRI score), which can combine the IR score and the SDRF score.

[0079] The longer the time period of interest, the more important that IR is to the risk of diabetes relative to SDRF. In some particular embodiments, one or both of these discrete scores can be "weighted" to correlate to the time frame of risk of interest. For example, the component parts of IR (e.g., eLP-IR) and SDRF can be weighted to have an increased value over the measured SDRF value to generate the DRI score. An equation to generate the DRI score is shown below.

$$\text{DRI score} = X\text{IR} + Y\text{SDRF} \qquad \textbf{EQUATION 1}$$

[0080] The DRI score can be a combination of the IR score and the SDRF score, and X and Y are defined coefficients where X>Y for LTR DRI evaluations. For example, X can be about 70% and Y can be about 30% for a 5 year risk of conversion timeline. X and Y can have other values. **Table 2** includes examples of relative sets of weights for a 5 year risk of conversion to DRI score, which can have a maximum value of 1, 10 or 100, in some embodiments. It will be apparent, that the SDRF score can decrease in relevance to the DRI risk as the evaluation window/risk period increases.

TABLE 2 IR AND SDRF WEIGHTS

DRI MAXIMUM	X%	Y%
1, 10, 100	70	30
10	75	25
100	60	40

[0081] In some embodiments, the analysis can use diabetes conversion data from one or more study populations that has a 5 year (or other appropriate) observation period to run a logistic regression analysis that includes the following prediction variables: age, gender, race, fasting glucose, IR (e.g., eLP-IR or other IR measure) and SDRF. From this prediction model, the coefficients for IR and SDRF can be generated. Then DRI can be calculated as a defined coefficient (X) times IR (e.g., eLP-IR) plus a defined coefficient (Y) times SDRF, per equation (1). The longer the timeframe for the

analysis, the more dominant IR scores will be relative to the SDRF scores. That is, the beta coefficients will provide the numbers for the associated statistical relationships which will not require any further weighting.

[0082] Embodiments of the invention provide clinical outputs of the DRI score as well as one or both of the two multi-marker parameters that can be combined to produce DRI score: the IR parameter (e.g., the eLP-IR score) which may be addressable by diet, exercise & weight loss and/or insulin sensitizing drugs, and the beta-cell dysfunction part, SDRF, which may be addressable potentially by drugs directed to this dysfunction. Another reason to measure and report an elevated SDRF is to alert the patient that diabetes is likely in their short-term future and therefore of more urgency to do something about (by weight loss at a minimum plus drugs).

[0083] Embodiments of the invention can employ STR scores to identify patients that may benefit from a drug therapy to improve or stabilize beta cell function.

[0084] Embodiments of the invention can employ STR scores to evaluate drug therapies, clinical trials and/or facilitate drug discovery.

[0085] The term "patient" is used broadly and refers to an individual that provides a biosample for testing or analysis.

[0086] The term "GlycA" refers to a biomarker that can be derived from a measure of composite NMR signal from carbohydrate portions of acute phase reactant glycoproteins containing N-acetylglucosamine and/or N-acetylgalactosamine moieties, more particularly from the protons of the 2-NAcGlc and 2-NAcGal methyl groups. The GlycA signal is centered at about 2.00 ppm in a plasma NMR spectrum at about 47 degrees C (+/-0.5 degrees C). The peak location is independent of spectrometer field but may vary depending on analysis temperature of the biosample and is not found in urine biosamples. Thus, the GlycA peak region may vary if the temperature of the test sample varies. **Figure 8** illustrates an NMR spectrum of a GlycA peak region (over an enlarged view of the region) according to embodiments of the present invention.

[0087] The GlycA NMR signal may include a subset of NMR signal at the defined peak region so as to include only clinically relevant signal contributions and may exclude a protein contribution to the signal in this region as will be discussed further below.

[0088] As used herein, the chemical shift locations (ppm) refer to NMR spectra referenced internally to CaEDTA signal at 2.519 ppm. Thus, the noted peak locations discussed and/or claimed herein may vary depending on how the chemical shift is generated or referenced as is well known to those of skill in the art. Thus, to be clear, certain of the described and/or claimed peak locations have equivalent different peak locations in other corresponding chemical shifts as is well known to those of skill in the art.

[0089] The term "biosample" refers to *in vitro* blood, plasma, serum, CSF, saliva, lavage, sputum, or tissue samples of humans or animals. Embodiments of the invention may be particularly suitable for evaluating human blood plasma or serum biosamples, particularly for GlycA (which is not found in urine, for example). The blood plasma or serum samples may be fasting or non-fasting. Where glucose is measured by NMR, the biosample is typically fasting blood plasma or serum samples. However, glucose may be measured by any suitable means.

[0090] The terms "population norm" and "standard" refer to values defined by a large study or studies such as the Framingham Offspring Study or the Multi-Ethnic Study of Atherosclerosis (MESA) or other study having a large enough sample to be representative of the general population. However, the instant invention is not limited to the population values in MESA or Framingham as the presently defined normal and at-risk population values or levels may change over time. Thus, a reference range associated with values from a defined population in risk segments (e.g., quartiles or quintiles) can be provided and used to assess elevated or reduced levels and/or risk of having a clinical disease state.

[0091] As used herein, the term "NMR spectral analysis" means using proton (^{1}H) nuclear magnetic resonance spectroscopy techniques to obtain data that can measure the respective parameters present in the biosample, e.g., blood plasma or blood serum.

[0092] "Measuring" and derivatives thereof refer to determining a level or concentration and/or for certain lipoprotein subclasses which can include measuring the average particle size thereof.

[0093] The term "NMR derived" means that the associated measurement is calculated using NMR signal/spectra from one or more scans of an *in vitro* biosample in an NMR spectrometer.

[0094] The term "lipoprotein component" refers to a lipoprotein component in a mathematical risk model associated with lipoprotein particles including size and/or concentration of one or more subclasses (subtypes) of lipoproteins. Lipoprotein components can include any of the lipoprotein particle subclasses, concentrations, sizes, ratios and/or mathematical products (multiplied) of lipoprotein parameters and/or lipoprotein subclass measurements of defined lipoprotein parameters or combined with other parameters such as GlycA.

[0095] The term "interaction parameter" refers to at least two different defined patient parameters combined (multiplied) as a mathematical product and/or ratio, typically one of the parameters is a sub-population of HDL-P and the other is an inflammatory marker. Examples of interaction parameters include, but are not limited to a sub-population of HDL, e.g., medium HDL-P ("HMP")/total HDL-P, (HMP)(GlycA), (HMP)(HZ), and/or a ratio of an inflammatory marker to a defined lipoprotein subpopulation, e.g., GlycA to HMP. The term "HZ" refers to average HDL-size. GlycA is an inflammatory biomarker. Other inflammatory biomarkers may be used in the interaction parameter, e.g., CRP (C-reactive

protein), hs-CRP (high-sensitivity CRP), IL-6 (interleukin-6), fibrinogen, haptoglobin, and alpha-1-acid glycoprotein.

[0096] The terms "mathematical model" and "model" are used interchangeably and when used with STR and/or DRI ("diabetes risk index") refers to a statistical model of risk used to evaluate a subject's risk of developing type 2 diabetes in a future time period or when used with IR, refers to a risk from a model of insulin resistance (a good predictor of diabetes risk) based on one or more study populations. The STR is for a time period that is shorter than that of a longer term risk (LTR). The risk models can be or include any suitable model including, but not limited to, one or more of a logistic model, a mixed model or a hierarchical linear model. The STR and DRI risk models can provide a measure of risk based on the probability of conversion to type 2 diabetes within a defined time frame, typically within 0-3 years for STR and greater than 3 years for LTR. The STR/SDRF risk model can be for a timeframe that is less than 3 years, such as within 6 months, 1 year, 1.5 years, 2 years, 2.5 years or 3 years, post test. The IR risk can be an important risk parameter for both STR and LTR longer term risk, e.g., greater than 3 years, such as 3.5 years, 4 years, 5 years or between 5-10 years, post test.

[0097] The DRI time period can be between about 5-7 years, more typically about 5 years. The STR, IR and/or DRI risk models can stratify a risk of developing T2DM as measured by standard $\chi 2$ and/or p values (the latter with a sufficiently representative study population).

Table 3: MESA Follow-up Periods

	Time from Baseline Exam (years)		
MESA Visit Number	**Mean**	**Minimum**	**Maximum**
2	**1.6**	**0.9**	**3.4**
3	**3.2**	**2.1**	**4.9**
4	**4.8**	**3.3**	**6.7**
5	**9.5**	**8.1**	**11.2**

[0098] **Table 3** shows the follow-up times for the different MESA visits (follow-up exams). The STR parameter can be derived from a regression model to predict diabetes diagnosed at visit 2. Mean follow-up time was 1.6 years (0.9 yr min; 3.4 yr max). **Figure 13** shows DM diagnosed at visits 2 or 3 in MESA. The IR parameter can have any timeframe from STR to LTR, while the SDRF is only relevant in the STR. In some embodiments, the IR model can be derived from a linear regression model predicting HOMA-IR (as shown in **Figure 12**).

[0099] It will be understood by those of skill in the art, almost all population studies conduct follow-up for some fixed period of time (e.g., 5 years) and do not monitor when during that time period diabetes was diagnosed. So the regression models are a cumulative measure, composed of some people who converted to diabetes sooner and others who converted later.

[0100] The term "LP-IR" score refers to a lipoprotein based insulin resistance score that rates a subject's insulin sensitivity from insulin sensitive to insulin resistant using a summation of risk scores associated with different defined lipoprotein components. *See, e.g.,* U.S. Patent No. 8,386,187 and Shalaurova I et al., Lipoprotein Insulin Resistance Index: A Lipoprotein Particle-Derived Measure of Insulin Resistance, Metabolic Syndrome and Related Disorders, Vol. 12, No. 8, Oct. 2014, pp. 422-429, for a detailed discussion of the LP-IR score. Generally stated, large VLDL, VLDL size, and small LDL have a positive risk association while large HDL, LDL size and HDL size have a negative association. These six components can be used to generate the LP-IR score (see, e.g., the bottom row of components indicated as associated with LP-IR in **Figure 2B**), which is typically a score between 1-100, with the risk scores of individual components varying as described in Table 3 of U.S. Patent No. 8,386,187. The LP-IR score can be calculated using NMR derived measurements of lipoproteins or other measurement methodologies.

[0101] Embodiments of the invention can employ risk models that include biomarkers that link to diabetic pathophysiology, including two or more of: insulin resistance, impaired β-cell function or impaired insulin secretion, inflammation and defective non-insulin (NI) dependent glucose uptake.

[0102] The role of HDL is complex and HDL-C is considered to be a relatively crude biomarker. Recently, researchers have suggested that HDL is an active player in diabetic pathophysiology rather than a bystander. *See,* Drew et al., The Emerging Roles of HDL in Glucose Metabolism, Nat. Rev., Endocrinol., 8, 237-245 (2012) published online 24 Jan. 2012.

[0103] The glycemic trajectory from normal to diabetic can typically be characterized by a long period of fairly stable glucose levels during which increasing insulin resistance is compensated for by increased β-cell insulin secretion, followed by an abrupt increase, generally less than about 3 years before diabetes diagnosis brought on by loss of β-cell mass and function. *See, e.g.,* Tabak AG et al., Lancet 2009: 373: 2215-21. Short term converters to diabetes are likely to have insulin resistance and beta-cell dysfunction or impairment. Markers associated with IR predict incident diabetes irre-

spective of the time frame of conversion. However, markers that, independent of IR and glucose, enhance prediction of short term conversion do not independently enhance prediction of longer term conversions provided by the IR markers.

[0104] Referring to **Figure 2A,** embodiments of the invention provide diabetes risk evaluations using at least two separate multi-markers of lipoprotein and metabolic parameters. The two multi-marker parameters include defined lipoprotein and metabolic parameters. The two multi-marker parameters are shown as (i) an IR risk factor score for insulin resistance that includes a plurality of defined lipoprotein and metabolic parameters and (ii) a STR or SDRF risk factor score for β-cell dysfunction that includes a plurality of defined lipoprotein and metabolic parameters, some of which can overlap with the parameters used for the STR risk factor score. The STR risk factor score can include a combination of (e.g., sum of) a plurality of separate values associated with a plurality or all of the following components: medium HDL-P, an inflammatory marker and an interaction parameter. The interaction parameter can be the inflammatory marker and a defined sub-population of HDL-P, typically med HDL-P.

[0105] The IR risk factor can include only the IR index or can include the IR index and other defined lipoprotein and metabolic parameters. For example, the IR risk factor score can be generated using a combination of separate values or scores of a plurality of or all of the following components: (a) an inflammatory marker, (b) med HDL-P, (c) med-VLDL-P, (d) one or more branched chain amino acids (BCAA) and (e) an insulin resistance index with a defined numerical range. The BCAA can include one or more of Valine, Leucine and Isoleucine, which may be measured by NMR or other methodologies. *See, e.g.,* PCT/US2013/064142 (WO 2014/059025), for a discussion of NMR measurement of BCAAs.

[0106] The IR risk factor can be the LP-IR or the eLP-IR (discussed further below) or other suitable insulin resistance index. For example, an insulin resistance index using metabolites identified in US 2009/0155826 to Hu et al. ("Hu"). Hu proposes the use of biomarkers to evaluate insulin resistance using biomarkers in one or more of Tables 4, 5, 6, 7, 8, 9A, 9B, 27, 28 and 29 (para. 98). After the level of one or more biomarker is determined, the level may be compared to disease or condition reference levels to determine a rating for each of the one or more biomarkers. The ratings can be aggregated using any algorithm to create a score, "for example, an insulin resistance (IR) score, for the subject (para. 106). Paragraph 107 goes on to give an example of an IR score of 100 indicates Type-2 diabetes, while a score of less than 25 may indicate normal glucose tolerance.

[0107] Referring to **Figure 2B,** the two separate multimarkers of lipoprotein and metabolite parameters are shown as (i) an extended LP-IR insulin resistance ("eLP-IR") score for the IR risk factor score and (ii) short-term diabetes risk factors ("SDRF") score for the STR factor score. The SDRF score can include at least one interaction parameter, shown as GlycA x med HDL-P. The two separate risk factor scores of the multi-marker parameters can assess diabetes risk at any level of glycemia of *in vitro* biosamples. The biosamples can be fasting plasma biosamples.

[0108] While the eLP-IR index is shown using LP-IR by way of example, other insulin resistance indexes or scores with a defined range may be used.

[0109] The eLP-IR score is an extended version of the LP-IR score as it includes the LP-IR score (with the six noted lipoprotein components indicated by the components on the bottom of the chart within the lines extending from the LP-IR box) as well as additional defined components including, as shown, GlycA, med HDL-P, med VLDL-P, and Valine. Other BCAAs may be included or used instead of Valine, including, for example, Leucine and Isoleucine. GlycA is an inflammatory biomarker and other inflammatory biomarkers may be included or substituted for GlycA, such as, for example, fibrinogen, hs-CRP, CRP, IL-6 or haptoglobin.

[0110] The SDRF score can include med HDL-P and at least one inflammatory marker, shown as GlycA, as well as at least one interaction parameter. The interaction parameter is not used in the eLP-IR or LP-IR scores as it is not associated with longer term risk (it is not statistically associated with insulin resistance but is associated with diabetes risk). Again, a different inflammatory marker or interaction parameter may be used. Notably, the interaction parameter, e.g., the interaction of HDL-P_{MED} and GlycA is believed to reflect a relationship of these variables with β-cell dysfunction. The results provide epidemiologic support for recent evidence for multiple roles of HDL in diabetic pathophysiology and for the modulation of HDL functionality by inflammation. **Figure 9** graphically illustrates the modulation of HDL functionality, e.g., changing from negative to a positive risk factor as the level of the inflammation, as assessed by GlycA, increases. While shown as at about the 80th percentile of GlycA for MESA, a 65th percentile value was identified using IRAS, both at a level of about 350 to 360 μmol/L.

[0111] The mathematical models can use other clinical parameters such as gender, age, BMI, whether on hypertension medicine, glucose and the like.

[0112] While it is contemplated that the STR, IR and DRI parameters can be provided as numerical scores within a defined numerical range, with lower scores associated with lower risk and higher scores associated with higher risk of conversion to diabetes, the risk scores or indexes can be presented on a patient report in different manners. The STR, IR and DRI scores can be provided as a result expressed numerically or alphanumerically, typically comprising a numerical score on a defined scale or within a defined range of values. For example, in particular embodiments, the STR, IR and/or DRI scores can be provided as or include a score within a defined range, such as, for example, between 0 and 1, on the low end, to 10 or 100 on the high end. Examples of ranges include: 0-0.1, 0-1, 0-5, 0-10, 1-10, 0-24, 1-24, 0-100, 1-100, 10-100, 0-1000, 1-1000, 10-1000 and the like. Typically, the lowest number is associated with the least risk and

the higher numbers are associated with increased risk of developing T2DM in the future. The lower value in the range may be above "0" such as 1, 2, 3, 4 or 5 and the like, or may even be a negative number (e.g., -1, -2, -3, 4, -5 and the like). Other index examples, include, for example, alphanumeric indexes or even icons noting degrees of risk, including but not limited to, "LR1" (low risk), IR5 (intermediate risk) and "HR9" (high risk), terms such as "DRI positive", "DRI high", "DRI neutral", "DRI low", "DRI good", "DRI bad", "DRI watch" and the like.

[0113] The IR, STR or DRI scores can all be between 0-10 or 1-10.

[0114] The IR score and the SDRF scores can have a range that is between 0-100 or 1-100 and DRI score can have a range of 0-10 or 1-10 as the risk models can apply a defined small coefficient to the different components of the respective risk models, e.g., a value that is less than 0.001 to the IR score, typically about 0.009 (**Figure 10**).

[0115] As noted above, the STR, IR and DRI diabetes risk indexes or scores can be decoupled from glucose measurements. Thus, for example, one or more of the STR, IR or DRI scores can be calculated for patients as a screening test or to stratify risk independent of glucose. That is, the STR/IR or DRI can be used to stratify risk for patients with glucose levels below 110 mg/dL, e.g., between 80-110 mg/dL and/or can predict diabetes conversion independent of fasting glucose or other measure of glycemia.

[0116] To help understand the information provided by the two different measurements of SDRF and IR, instructional guidelines and/or an electronic program can be provided to a clinician that generates a test result when both data points are supplied. The combined data evaluation can be provided as a download from a laboratory or from an offering company, such as, for example, LipoScience (Raleigh, NC). Instructional guidelines can be provided to a clinician so that the clinician can understand the risk stratification provided by the STR and/or DRI scores and can inform a clinician whether to order a glucose challenge test which may be more time consuming, expensive or inconvenient for a patient. An electronic risk analysis circuit can also be provided (e.g., a portal accessible via the Internet) that can generate risk information based on STR, IR and/or DRI scores.

[0117] The STR/SDRF, IR and/or DRI scores can be generated independent of and/or without requiring concurrent glucose measurements and may be used to allow a clinician to consider what risk category a respective patient may belong to.

[0118] Lipoproteins include a wide variety of particles found in plasma, serum, whole blood, and lymph, comprising various types and quantities of triglycerides, cholesterol, phospholipids, sphyngolipids, and proteins. These various particles permit the solublization of otherwise hydrophobic lipid molecules in blood and serve a variety of functions related to lipolysis, lipogenesis, and lipid transport between the gut, liver, muscle tissue and adipose tissue. In blood and/or plasma, lipoproteins have been classified in many ways, generally based on physical properties such as density or electrophoretic mobility or measures of apolipoprotein A-1 (Apo A-1), the main protein in HDL.

[0119] Classification based on nuclear magnetic resonance-determined particle size distinguishes distinct lipoprotein particles based on size or size ranges. For example, the NMR measurements can identify at least 15 distinct lipoprotein particle subtypes, including at least 5 subtypes of high density lipoproteins (HDL), at least 4 subtypes of low density lipoproteins (LDL), and at least 6 subtypes of very low density lipoproteins (VLDL), which can be designated TRL (triglyceride rich lipoprotein) V1 through V6.

[0120] The NMR derived estimated lipoprotein sizes, e.g., HDL-P particle sizes for H1-H26, are not exact but are approximate to estimate each subclass to a size range. Other methodologies may provide different size ranges that correlate to the NMR estimated subclass sizes.

[0121] Further, it is also noted that while NMR measurements of the lipoprotein particles are particularly suitable for the analyses described herein, it is contemplated that other technologies may be used to measure these parameters now or in the future and embodiments of the invention are not limited to this measurement methodology. It is also contemplated that different protocols using NMR may be used (e.g., including different deconvolving protocols) in lieu of the deconvolving protocol described herein. *See, e.g.,* Kaess et al., The lipoprotein subfraction profile: heritability and identification of quantitative trait loci, J Lipid Res. Vol. 49 pp. 715-723 (2008); and Suna et al., 1H NMR metabolomics of plasma lipoprotein subclasses: elucidation of metabolic clustering by self-organizing maps, NMR Biomed. 2007; 20: 658-672. Flotation and ultracentrifugation employing a density-based separation technique for evaluating lipoprotein particles and ion mobility analysis are alternative technologies for measuring lipoprotein subclass particle concentrations. Vertical auto profile methodology or other subfractionation methods may potentially be used. *See,* Martin et al., High-density lipoprotein subfractions: Current views and clinical practice applications, Trends Endocrinol Metab, 2014; 25; 329-336.

[0122] Lipoprotein subclass grouping can include subclasses with concentrations that can be summed to determine VLDL-P, HDL-P and LDL-P numbers according to some particular embodiments of the present invention. It is noted that the "small, large and medium" size ranges noted can vary or be redefined to widen or narrow the upper or lower end values thereof or even to exclude certain ranges within the noted ranges. The particle sizes noted typically refer to average measurements, but other demarcations may be used. **Figures 3A** and **3B** illustrate exemplary size ranges for different sub-populations of lipoproteins. Embodiments of the invention classify lipoprotein particles into subclasses grouped by size ranges based on functional/metabolic relatedness as assessed by their correlations with lipid and

metabolic variables. Thus, as noted above, the evaluations can measure over 20 discrete subpopulations (sizes) of lipoprotein particles, typically between about 30-80 different size subpopulations (or even more).

[0123] For the GlycA measurement calculations, where used, the discrete number of HDL and LDL groupings can be less than those used to quantitatively measure the lipoprotein subclasses (where NMR is used for the lipoprotein measurements). The subclasses of different size can be quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. *See,* Jeyarajah et al., Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy, Clin Lab Med. 2006; 26: pp. 847-870.

[0124] The term "LDL-P" refers to a low density lipoprotein particle number (LDL-P) measurement (e.g., LDL-P number) that sums the concentration of defined LDL subclasses. Total LDL-P can be generated using a total low density lipoprotein particle (LDL-P) measurement that sums the concentration (μmol/L) of all the LDL subclasses (large and small) including sizes between 18-23 nm. In some embodiments, the LDL-P measurement may employ selected combinations of the LDL subclasses (rather than the total of all LDL subclass subpopulations). As used herein, the term "small LDL particles" typically includes particles whose sizes range from between about 18 to less than 20.5 nm, typically between 19-20 nm. The term "large LDL particles" includes particles ranging in diameter from between about 20.5-23 nm. It is noted that the LDL subclasses of particles can be divided in other size ranges. For example, the "small" size may be between about 19-20.5 nm, intermediate may be between about 20.5-21.2 nm, and large may be between about 21.2-23 nm. In addition, intermediate-density lipoprotein particles ("IDL" or "IDL-P"), which range in diameter from between about 23-29 nm, can be included among the particles defined as "large" LDL (or even small VLDL). Thus, for example, the LDL subclasses can be between 19-28 nm.

[0125] The term "HDL-P" refers to a high density lipoprotein particle number (HDL-P) measurement (e.g., HDL-P number) of a concentration of a defined sub-population of lipoprotein subclasses. Total HDL-P can be generated using a total high density lipoprotein particle (HDL-P) measurement that sums the concentration (μmol/L) of all the HDL subclasses (which may be grouped based on size into different size categories such as large, medium and small) in the size range between about 7 nm to about 15 nm, typically between 7.3 or 7.4 and 13.5 or 14 nm.

[0126] HDL subclass particles typically range from between about 7 nm to about 15 nm, more typically about 7.3 nm to about 14 nm (e.g., 7. 4 nm-13.5 nm). The HDL-P concentration, at least when measured by certain methodologies including NMR deconvolution, is the sum of the particle concentrations of the respective subpopulations of its HDL-subclasses. The HDL subpopulation can include medium HDL particle subclasses (HMP). Medium HDL-P, which is referred to interchangeably herein using the abbreviations "HMP" or "HDL-P_{MED}" or "med-HDL-P", refers to a defined sub-population of HDL particles that excludes small and large HDL particle subclasses. The exact size range of HMP/med-HDL-P/HDL-P_{MED} may vary between measurement methodologies and study populations.

[0127] However, as is well known to those of skill in the art, HMP/ med-HDL-P/HDL-P_{MED}, as well as the other lipoprotein subpopulations used in the different IR/STR risk models can be selected to maximize correlation of risk of conversion to diabetes using a risk model (typically a logistic regression model of at least one defined study population), either, or both, in the short term or longer term multi-parameter STR and IR risk models.

[0128] **Figure 5** graphically illustrates HDL subpopulations grouped by sizes and incident diabetes risk associations from MESA. As noted herein, the HDL sizes of the HDL-PMED subclass can vary and can depend on risk associations in a study population, this graph shows negative and positive associations with different groupings of sizes by way of example only. The graph illustrates diabetes risk associations for 9 different size groupings or sub-populations of the 26 HDL subpopulations with three boxes of further groupings of selected HDL subclasses according to embodiments of the present invention. The $\chi 2$ values from the logistic regression model indicate the strengths and signs of the risk associations as determined in the MESA study population during 6 years of follow-up among 4968 MESA participants with 411 incident cases of diabetes diagnosed (all 9 subpopulations were included in the same logistic regression model, adjusted for age, gender, race, and glucose).

[0129] In some embodiments, the different subpopulations of HDL-P can be identified by a number from 1-26, with "1" representing the smallest size subpopulation in the HDL class and "26" being the largest size subpopulation in the HDL class as shown in **Figure 4.**

[0130] HMP can include, for example, HDL particles with sizes corresponding to H9-H14, or H9-H15, and optionally H9-H17, of respective subpopulations. **Figure 4** illustrates the optional different exemplary subclass groupings that can be used to define the med-HDL-P subpopulation. These size categories can be selected to optimize risk stratification for individuals having an intermediate risk in a population norm based on (fasting) glucose measurements of 90 mg/dL (or even less) to 110 mg/dL. That is, the lipoprotein subclass sub-populations or groups for a particular parameter can be selected based on a statistical analysis of study populations such as MESA, IRAS and/or Framingham to determine how the various subpopulations should be grouped based on risk association with T2DM, rather than LP-IR or insulin resistance.

[0131] In some embodiments, medium HDL-P (HMP/HDL-P_{MED}) can be associated with HDL-P in the range of one of: 8.3-9.2 nm, 8.3-9.4 nm, 8.3-9.7 nm, 8.3 to 10 nm or 8.3 nm to 10.2 nm (estimated sizes based on NMR measurements).

[0132] Diabetes prediction performance in the IRAS study dataset was better with a somewhat narrower size range

and not much worse in the MESA study dataset (which yielded a medium HDL-P span of 8.3 nm to 10.0 nm). However the upper range for medium HDL-P can be 9.4 nm, 10.0 nm or 10.2 nm, or even slightly higher or lower, for example.

[0133] The term "large VLDL particles" refers to particles at or above 60 nm such as between 60-260 nm. The term "medium VLDL particles" ("medVLDL-P" or "VLDL-Pmed") refers to particles with sizes between 35-60 nm. The term "small VLDL particles" refers to particles between 29-35 nm. The term "VLDL-P" refers to a very low density lipoprotein particle number (VLDL-P) measurement (e.g., VLDL-P number) that sums the concentration of defined VLDL subclasses. Total VLDL-P can be generated by summing the concentrations (nmol/L) of all the VLDL subclasses (large, medium and small). The exact size range for medium and large VLDL may vary between measurement methodologies and study populations but each is associated with a defined sub-population of lipoproteins which can be defined based on risk associations of different sub-class groupings.

[0134] As noted above, embodiments of the present invention provide STR, IR and DRI scores using defined mathematical models of risk of different defined lipoprotein and metabolite biomarkers or parameters of *an in vitro* biosample(s) of a patient or subject to identify at-risk patients or subjects before onset of T2DM who may benefit from pharmaceutical, medical, diet, exercise or other intervention.

[0135] One or more of the STR, IR and/or DRI scores can also or alternatively be used during clinical trials and/or for drug discovery to monitor for positive or negative changes in metabolic or cellular function.

[0136] The STR, IR and/or DRI evaluation can be decoupled from a glucose measurement and can relatively easily be generated as a screening tool and may be able to identify at-risk individuals earlier in time than with conventional tests.

[0137] An "unprocessed biosample" as used herein refers to a biosample that, unlike sample preparation for mass spectrometry analysis, is not subjected to processing that causes the biosample to be physically or chemically altered after it is obtained (but buffers and diluents can be used). Thus, once the biosample is obtained, components from the biosample are not altered or removed. For example, once a blood serum biosample is obtained, the serum is not subjected to processing that removes components from the serum. In some embodiments, an unprocessed biosample is not subjected to filtering and/or ultrafiltration processes.

[0138] In some embodiments, a patient glucose measurement can be obtained via NMR analysis of the biosample NMR spectrum, along with lipoprotein particle measurements, GlycA and Valine measurements. However, glucose measurements, where used, can alternatively be obtained in conventional chemical ways.

[0139] In some particular embodiments, it is contemplated that NMR measurements of GlycA, Valine, and lipoproteins of a single (blood/plasma) *in vitro* biosample can be analyzed to provide important clinical information and/or further improve a prediction or evaluation of a patient or subject's risk of developing type 2 diabetes and/or having prediabetes in the future.

[0140] **Figure 6A** is a graph of diabetes conversion rate versus glucose category (low risk, intermediate risk, high risk) based on MESA 5-year T2DM conversion (n=359/4211) illustrating an unmet need for stratifying or better identifying patients at increased risk of progression to T2DM for intermediate risk glucose patients according to embodiments of the present invention. **Figure 6B** is a graph of diabetes conversion rate versus glucose category (low risk, intermediate risk, high risk) based on IRAS 5-year T2DM conversion (n=134/978) also illustrating an unmet need for stratifying or better identifying patients at increased risk of progression to T2DM for, particularly for those deemed to be in the low or intermediate risk glucose region according to embodiments of the present invention. **Figures 6A/6B** contrast the risk assigned on the basis of glucose level ("low" <100; "high" >=100 as implied by the designation of "prediabetes" for glucose levels in the elevated rage of 100-125) and actual risk shown on the y axis categorized (implicitly) as not high (<15% 5-year conversion), "high" 15-25%, and "very high" (>25%). In MESA, for example, 39 + 87 "low risk" people with glucose <100 developed diabetes, so their risk was not actually low. The DRI scores provided by embodiments of the invention can subdivide putatively "low risk" individuals into those with truly low risk and others with high or even very high risk (shown better by the wide ranges of predicted diabetes rates within each glucose category).

[0141] **Figure 7** is a chart of the characteristics of the MESA study population (n=3450).

[0142] **Figure 8** is a graph of NMR spectra showing the GlycA, Valine and lipoprotein subclasses peak regions.

[0143] **Figure 9** is a graph of predicted short term diabetes conversion rate by GlycA level (μmol/L) for three defined levels of HDL-P_{MED} (high, intermediate and low) illustrating high HDL-P_{MED} turns from "good" to "bad" at about an 80th percentile level of GlycA. The high HDL-PMED line is represented by the low line on the left side which rises to the higher risk top line on the right. The low HDL-P_{MED} line is shown with the stars on the line illustrating its decreasing risk association as GLycA levels rise (when inflammation increases).

[0144] The HDL-P_{MED} values can be 9, 12.9 and 17.17, representing 25th, 50th and 75th percentiles. The predicted probabilities for conversion to diabetes for short term conversion to diabetes in MESA (n=181/3450) can be based on the logistic regression for a prophetic 60 year old Caucasian female with fasting plasma glucose (FPG) =105 mg/dL and 50th percentile eLP-IR score (0.90).

[0145] The concentration range of GlycA is typically between about 220 to 500 μmol/L, inclusive thereof.

[0146] **Figure 10** is a block diagram similar to **Figure 2B,** illustrating exemplary mathematical equations with exemplary coefficients that may be used with the noted respective components to generate eLP-IR and SDRF scores. The eLP-

IR score can be combined with the SDRF score to generate a DRI score. In some embodiments, each score can be provided or one of the different scores can be provided to a user for evaluation.

[0147] The eLP-IR score typically ranges from about 0.1 to 1.8 and can be reported to a patient and/or clinician in percentile values using a population norm such as MESA as a reference population.

[0148] The SDRF score typically ranges between from about -6.4 to -1.6 and can also be reported in percentile units.

[0149] The DRI score is typically transformed for reporting purposes to have a range between 1 and 10. That is, the exemplary the ranges given here are for the "raw" output values of eLP-IR and SDRF, i.e., the values produced by the 2 equations at the bottom of the **Figure 10** pyramid. For reporting purposes, the SDRF and eLP-IR values can be mathematically altered/transformed into percentile values (giving 1-100 scores, for example).

[0150] **Figure 11A** is a graph showing vertical rectangles of with ranges of DRI-predicted diabetes conversion rates (%) for participants in the MESA study within the glucose categories of <95 mg/dL, 95-99 mg/dL and 100-110 mg/dL, respectively, to illustrate how the DRI score can stratify the risk of persons within the same narrow glucose range.

[0151] **Figure 11B** is a similar graph showing the ranges of DRI-predicted diabetes conversion rates (%) for participants in the IRAS study within the glucose categories of <95 mg/dL, 95-99 mg/dL and 100-110 mg/dL according to embodiments of the present invention, which validates that MESA derived DRI risk models can stratify a person's risk within the same glucose range.

[0152] **Figure 12** is a chart showing the parameters included in three linear regression models predicting insulin resistance (as assessed by HOMA-IR) in MESA (n=3446). Strengths of association of each parameter with insulin resistance are given as the difference in ln (HOMA-IR) per one standard deviation increment. eLP-IR can be represented by Model 2 parameters with the noted exemplary beta-coefficients in **Equation 2A** contributing to the eLP-IR multimarker parameter of insulin resistance according to embodiments of the present invention. **Equation 2B** is a more generalized version of **Equation 2A** recognizing the coefficient values (shown by A-E) can vary from those shown in **Equation 2A** (based on a different study population and/or on a different inflammatory marker, different IR or a different BCAA parameter, for example).

$$\text{eLP-IR} = (0.00935)\,(\text{LP-IR}) + (0.001687)\,(\text{Valine}) - (0.002594)\,(\text{VLDL-P}_{\text{MED}}) - (0.01096)\,(\text{HDL-P}_{\text{MED}}) + (0.000848)\,(\text{GlycA}) \qquad \textbf{EQUATION 2A}$$

$$\text{eLP-IR} = (A)\,(\text{LP-IR}) + (B)\,(\text{Valine}) - (C)\,(\text{VLDL-P}_{\text{MED}}) - (D)(\text{HDL-P}_{\text{MED}}) + (E)(\text{GlycA})$$

EQUATION 2B

[0153] **Figure 13** is a chart of parameters contributing to the SDRF multimarker parameter of short term risk according to embodiments of the present invention, from logistic regression in MESA with short-term (n=181/3450) or long-term (n=286/3269) diabetes conversion as dependent variable. Strengths of association of each parameter in the different models are given by the odds ratio (OR) per one standard deviation increment. SDRF was derived using **Equation 3A** using beta-coefficients from Model 4 for short-term diabetes conversion. **Equation 3B** is a more generalized version of **Equation 3A** recognizing the coefficient values (shown by A-C) can vary from those shown in **Equation 3A** (based on different study populations and/or on a different inflammatory marker, or a different interaction parameter, for example).

$$\text{SDRF} = -\,(0.352)\,(\text{HDL-P}_{\text{MED}}) - (0.0108)\,(\text{GlycA}) + (0.000969)\,(\text{GlycA x HDL-P}_{\text{MED}})$$

EQUATION 3A

$$\text{SDRF} = -\,(A)\,(\text{HDL-P}_{\text{MED}}) - (B)\,(\text{GlycA}) + (C)\,(\text{GlycA x HDL-P}_{\text{MED}}) \qquad \textbf{EQUATION 3B}$$

[0154] The "raw" eLP-IR and SDRF risk scores from **EQUATIONS 2A** and **3A,** respectively, can be transformed for reporting purposes into respective scores in a range between 1-100 based on percentile values within a reference population such as MESA. In such case of eLP-IR, values <0.7 (<25th percentile) and >1.1 (≥75th percentile) could signify a low and high risk, respectively, for developing type 2 diabetes. In the case of SDRF, values <-4.1 (<25th percentile) and >-3.8 (≥75th percentile) could indicate a low and high risk, respectively, of converting to diabetes within a relatively short time period. Examples of ranges of raw scores for SDRF and eLP-IR are provided in **Table 4** below.

Table 4 Percentile Values in MESA for eLP-IR and SDRF scores

	Minimum	Maximum	25th percentile	50th percentile	75th percentile
eLP-IR	0.1	1.7	0.7	0.9	1.1
SDRF	-6.4	-1.6	-4.1	-3.9	-3.8

[0155] The defined HDL-P subpopulation for IR (e.g., eLP-IR) may differ from the HDL-P subpopulation used for SDRF but is typically the same concentration measurement of the same defined HDL-P subpopulation used to calculate the SDRF score.

[0156] SDRF and eLP-IR scores or parameters can be combined into a composite or cumulative DRI score. In some embodiments, the SDRF and IR score (e.g., eLP-IR) coefficients (weights) can be selected/defined using a logistic regression model for 5-year diabetes conversion in people with glucose <110 mg/dL. MESA or other data can be used for that purpose. The selected weighting factors can be validated in a different study in assessing 5-year risk in IRAS.

[0157] Logistic models in both MESA (5-y) and IRAS (5-y) as well as MESA longer-term (~10-y) were generated to provide examples of coefficients and chi-square values for SDRF and eLP-IR in those 3 situations. These are only examples as the eLP-IR and/or SDRF components, e.g., Valine or GlycA assays may be adjusted or other IR models/scores, other inflammatory markers or other BCAAs may be used, for example. As is well known to those of skill in the art, the logistic models can be run with the different components to select/define the coefficients. **Table 5** is a chart showing exemplary coefficients that can be used to provide the X and Y factors (Equation 1) for the DRI scores, with SDRF having almost no value in the 10 year conversion.

Table 5 Exemplary X and Y coefficients for DRI scores.

MESA 5-year conversion among participants with glucose <110 mg/dL (n=270/4751)						
Model	Parameter	Coefficient	Model χ^2	AUC	Parameter χ^2	p
age, sex, race, glucose	---	---	296.2	0.774	---	---
+eLP-IR+SDRF	e-LP-IR	2.1017	360.2	0.805	46.6	<0.0001
	SDRF	0.5163			6.55	0.01
MESA 10-year conversion among participants with glucose <110 mg/dL (n=362/3319)						
Model	Parameter	Coefficient	Model χ^2	AUC	Parameter χ^2	p
age, sex, race, glucose	---	---	328.0	0.766	---	---
+eLP-IR+SDRF	e-LP-IR	2.4475	411.8	0.801	74.0	<0.0001
	SDRF	0.0391			0.04	ns
IRAS 5-year conversion among participants with glucose <110 mg/dL (n=88/844)						
Model	Parameter	Coefficient	Model χ^2	AUC	Parameter χ^2	p
age, sex, race, glucose	---	---	48.55	0.715	---	---
+eLP-IR+SDRF	e-LP-IR	2.2294	79.7	0.776	14.7	0.0001
	SDRF	0.7292			13.0	0.0003

[0158] In some embodiments, the "raw" DRI values can range from about -3.0 to about +1.8, but other raw score ranges are possible. The DRI raw scores may optionally be transformed into a defined standardized score range of 1-10 or 0-10, for example.

[0159] Thus, for example, in some embodiments, using the first set of coefficients (MESA 5-year) the DRI equation can be: DRI = 2.1017 (eLP-IR) + 0.5163 (SDRF).

[0160] In some embodiments, rather than using percentiles, the DRI range can be segmented into up into a set of equal parts, e.g., 10-50, typically 20, equal parts to transform the raw values into DRI score values ranging from 1-10 in 0.5 increments (i.e., 0.5, 1.0, 1.5, ...10) to provide the risk assessment, each with larger values representing increased risk over lower values.

[0161] **Figure 14** is a chart illustrating the conversion to diabetes during a 5.2 year follow-up in IRAS (n=134/976) which validates the model developed using MESA according to embodiments of the present invention. The data is based

on logistic regression in IRAS with 5-year diabetes conversion (actually 5.2 year conversion) as the dependent variable. Relative predictive values of the 5 regression models are given by the likelihood ratio (LR) χ^2 statistic and area under the ROC curve (AUC). Strengths of association of the indicated variables are given by the odds ratio (OR) per one standard deviation increment. S_i is insulin sensitivity measured by frequently sampled intravenous glucose tolerance testing. **Figure 14** also shows the improved diabetes risk association provided by eLP-IR relative to the prior LP-IR (124.2 versus 119.9) LRχ^2.

[0162] **Figure 15** is a chart illustrating the meaning of the interaction parameter based on observed (not predicted) 5-year diabetes conversion rates in 9 subgroups of IRAS participants (all with fasting glucose less than or equal to 110 mg/dL) categorized by their levels (low, intermediate or high by tertile) of GlycA and HDL-P_{MED} according to embodiments of the present invention. For example, when GlycA level is low (bottom tertile), HDL-P_{MED} levels are strongly inversely related to diabetes risk (rates of 2.3% vs 10.1% when HDL-P_{MED} is high vs low, respectively). However, when GlycA level is high (upper tertile), having a high level of HDL-P_{MED} is not good, but actually worse (17.8% conversion rate) than having a low HDL-P_{MED} level (12.9% rate).

[0163] **Figure 16** is an exemplary graph of fasting glucose (mg/dL) over time (years) for hypothetical patients with high or low levels of insulin resistance (IR) and SDRF score to illustrate that SDRF and IR (eLP-IR, for example) scores can be used with intermediate or low glucose values to predict whether the glucose value will be stable over time or have a trajectory that leads to diabetes in the future, e.g., either in the short term or longer term according to embodiments of the present invention. For example, when both the SDRF score and the IR score are indicated as elevated (e.g., above the 75th percentile), then the glucose trajectory is more likely to rise over a relatively short timeframe, e.g., about 2-3 years or less from the time of the test.

[0164] **Figure 17** is an exemplary report which provides a measure of short term risk, e.g., a SDRF score, which may be monitored for change to assess β-cell function/dysfunction over time and provided as a reference or historical segment on the report. A raw STR score in the third tertile or above, e.g., ≥-3.8 (≥75th percentile) could indicate a high risk of converting to diabetes within a relatively short time period, e.g., two years or less post-test.

[0165] **Figures 20A-20C** are also exemplary reports which provide the same DRI scores with different glucose levels. The reports can also provide separate scores for the inflammatory marker (e.g., GlycA) and a BCAA, e.g., Valine.

[0166] The DRI score can also be provided with an associated 5-year risk category. The 5-year risk timeline is believed to be of clinical significance to patients, potentially causing or prompting the patient to take a more active response, e.g., diet, exercise or drug therapy relative to longer timelines. However, longer timelines may be used. The DRI score (e.g., Diabetes Risk Index) can be provide alone or with an interpretation segment with a graph of risk relative to a FPG score of the patient. The historical reporting can correlate the STR or DRI score to the FPG score. Even if the FPG score remains relatively level, the STR, IR or DRI score can indicate a progression toward diabetes if these scores increase.

[0167] **Figures 20A-20C** illustrate that patients having the same 7.5 DRI score can have different risks, shown as, very high, high and low 5-year risk category, respectively, based on their FPG level.

[0168] **Figure 18** is a schematic illustration of an exemplary patient report that can provide one or more of a DRI score, an IR score (associated with insulin resistance) and an STR score (associated with β-cell function) with other lipoprotein and/or metabolite parameters (e.g., the inflammatory marker) according to embodiments of the present invention.

[0169] **Figure 19** is an exemplary graph that can be used to evaluate change in one or more of a DRI score, an IR score or an STR scores over time, which may be based or correlated to doses or types of therapies according to embodiments of the present invention.

[0170] The GlycA levels may be measured by NMR in "arbitrary units" that may be converted to methyl group concentration units (umol/L) by multiplying by 17.8.

[0171] Referring now to **Figure 21,** it is contemplated that in some particular embodiments, most, if not all, the measurements for STR, IR and/or DRI scores can be carried out on or using a system **10** in communication with or at least partially onboard an NMR clinical analyzer **22** as described, for example, with respect to **Figure 22** below and/or in U.S. Patent No. 8,013,602.

[0172] The system **10** can include a STR, IR and/or DRI diabetes risk index (e.g., score) Module **370** to collect data suitable for determining one or all components of the risk scores (e.g., HDL subpopulations, GlycA, Valine). The system **10** can include an analysis circuit **20** that includes at least one processor **20p** that can be onboard the analyzer **22** or at least partially remote from the analyzer **22.** If the latter, the Module **370** and/or circuit **20** can reside totally or partially on a server **150.** The server **150** can be provided using cloud computing which includes the provision of computational resources on demand via a computer network. The resources can be embodied as various infrastructure services (e.g. computer, storage, etc.) as well as applications, databases, file services, email, etc. In the traditional model of computing, both data and software are typically fully contained on the user's computer; in cloud computing, the user's computer may contain little software or data (perhaps an operating system and/or web browser), and may serve as little more than a display terminal for processes occurring on a network of external computers. A cloud computing service (or an aggregation of multiple cloud resources) may be generally referred to as the "Cloud". Cloud storage may include a model of networked computer data storage where data is stored on multiple virtual servers, rather than being hosted on one or

more dedicated servers. Data transfer can be encrypted and can be done via the Internet using any appropriate firewalls to comply with industry or regulatory standards such as HIPAA. The term "HIPAA" refers to the United States laws defined by the Health Insurance Portability and Accountability Act. The patient data can include an accession number or identifier, gender, age and test data.

[0173] The results of the analysis can be transmitted via a computer network, such as the Internet, via email or the like to a patient, clinician site **50**, which may include an electronic display **50D**, to a health insurance agency **52** or a pharmacy **51** and/or the patient **53.** The results can be sent directly from the analysis site or may be sent indirectly. The results may be printed out and sent via conventional mail. This information can also be transmitted to pharmacies and/or medical insurance companies, or even patients that monitor for prescriptions or drug use that may result in an increase risk of an adverse event or to place a medical alert to prevent prescription of a contradicted pharmaceutical agent. The results can be sent to a patient via email to a "home" computer or to a pervasive computing device such as a smart phone or notepad and the like. The results can be as an email attachment of the overall report or as a text message alert, for example.

[0174] Still referring to **Figure 21,** one or more electronic devices **50D, 51D, 53D, 60D** associated with the different users, e.g., a clinician site **50,** patient **52D** and/or a test or lab site **60** can be configured to access an electronic analysis circuit **155** in communication with a display of a respective electronic device. The analysis circuit **155** can be hosted on a server **150** and can provide an internet portal or downloadable APP or other computer program for various devices. The circuit **155** can configured to allow a user, e.g., a clinician to enter one or more of: (i) a glucose value of a patient, (ii) a glucose value of a patient and a diabetes risk index score, or (iii) a diabetes risk index score. The circuit can automatically populate different data fields based on a patient identifier or other password at sign-in or allow a user to enter one or more of a STR, IR or DRI score and a glucose measurement for a respective patient. The analysis circuit can be configured to track changes in the STR, IR and/or DRI score over time and generate electronic reports that can be sent to clinicians, patients or other users. The analysis circuit can also send notices for recommendations on retests, follow-up tests and the like, e.g., if a STR, IR or DRI risk score is elevated or above a low risk value, e.g., in an intermediate risk category, the circuit can notify the clinician that a glucose test may be appropriate or send a notice to the patient to confer with the doctor to see if a glucose test is appropriate or whether increased monitoring intervals for follow-on DRI tests may be desirable.

[0175] The analysis circuit **155** and/or **20** can generate a risk progression pathway or analysis to provide graphic information that stratifies risk of developing type 2 diabetes in the future for patients having the same glucose value when the glucose value is in an intermediate risk range, when fasting plasma glucose levels are between 90-110 mg/dL, A1C % levels are between 5.7-6.4 or oral glucose tolerance levels are between 140-199 mg/dL. The electronic analysis circuit can be onboard the server **150** in the Cloud or otherwise accessible via the Internet **227** or can be associated with a different client architecture as will be appreciated by one of skill in the art. Thus, a clinician, patient or other user can generate a customized report on risk progression or otherwise obtain risk stratification information.

[0176] Referring now to **Figure 22,** a system **207** for acquiring at least one NMR spectrum for a respective biosample is illustrated. The system **207** includes an NMR spectrometer **22s** and/or analyzer **22** for obtaining NMR data for NMR measurements of a sample. In one embodiment, the spectrometer **22s** is configured so that the NMR signal acquisition is conducted at about 400 MHz for proton signals; in other embodiments the measurements may be carried out at between about 200MHz to about 900 MHz or other suitable frequency. Other frequencies corresponding to a desired operational magnetic field strength may also be employed. Typically, a proton flow probe is installed, as is a temperature controller to maintain the sample temperature at 47 +/- 0.5 degrees C. The spectrometer **22s** can be controlled by a digital computer **214** or other signal processing unit. The computer **211** should be capable of performing rapid Fourier transformations. It may also include a data link **212** to another processor or computer **213,** and a direct-memory-access channel **214** which can connects to a hard memory storage unit **215** and/or remote server **150 (Figure 15).**

[0177] The digital computer **211** may also include a set of analog-to-digital converters, digital-to-analog converters and slow device I/O ports which connect through a pulse control and interface circuit **216** to the operating elements of the spectrometer. These elements include an RF transmitter **217** which produces an RF excitation pulse of the duration, frequency and magnitude directed by the digital computer **211,** and an RF power amplifier **218** which amplifies the pulse and couples it to the RF transmit coil **219** that surrounds sample cell **220** and/or flow probe **220p.** The NMR signal produced by the excited sample in the presence of a 9.4 Tesla polarizing magnetic field produced by superconducting magnet **221** is received by a coil **222** and applied to an RF receiver **223.** The amplified and filtered NMR signal is demodulated at **224** and the resulting quadrature signals are applied to the interface circuit **216** where they are digitized and input through the digital computer **211.** The diabetes risk evaluation Module **370** or analysis circuit **20, 155 (Figures 21)** or can be located in one or more processors associated with the digital computer **211** and/or in a secondary computer **213** or other computers that may be on-site or remote, accessible via a worldwide network such as the Internet **227.**

[0178] After the NMR data are acquired from the sample in the measurement cell **220,** processing by the computer **211** produces another file that can, as desired, be stored in the storage **215.** This second file is a digital representation of the chemical shift spectrum and it is subsequently read out to the computer **213** for storage in its storage **225** or a

database associated with one or more servers. Under the direction of a program stored in its memory, the computer **213,** which may be a personal, laptop, desktop, workstation, notepad, tablet or other computer, processes the chemical shift spectrum in accordance with the teachings of the present invention to generate a report which may be output to a printer **226** or electronically stored and relayed to a desired email address or URL. Those skilled in this art will recognize that other output devices, such as a computer display screen, notepad, smart phone and the like, may also be employed for the display of results.

[0179] It should be apparent to those skilled in the art that the functions performed by the computer **213** and its separate storage **225** may also be incorporated into the functions performed by the spectrometer's digital computer **211.** In such case, the printer **226** may be connected directly to the digital computer **211.** Other interfaces and output devices may also be employed, as are well-known to those skilled in this art.

[0180] Embodiments of the present invention may take the form of an entirely software embodiment or an embodiment combining software and hardware aspects, all generally referred to herein as a "circuit" or "module."

[0181] As will be appreciated by one of skill in the art, the present invention may be embodied as an apparatus, a method, data or signal processing system, or computer program product. Accordingly, the present invention may take the form of an entirely software embodiment, or an embodiment combining software and hardware aspects. Furthermore, certain embodiments of the present invention may take the form of a computer program product on a computer-usable storage medium having computer-usable program code means embodied in the medium. Any suitable computer readable medium may be utilized including hard disks, CD-ROMs, optical storage devices, or magnetic storage devices.

[0182] The computer-usable or computer-readable medium may be, but is not limited to, an electronic, magnetic, optical, electromagnetic, infrared, or semiconductor system, apparatus, device, or propagation medium. More specific examples (a non-exhaustive list) of the computer-readable medium would include the following: an electrical connection having one or more wires, a portable computer diskette, a random access memory (RAM), a read-only memory (ROM), an erasable programmable read-only memory (EPROM or Flash memory), an optical fiber, and a portable compact disc read-only memory (CD-ROM). Note that the computer-usable or computer-readable medium could even be paper or another suitable medium, upon which the program is printed, as the program can be electronically captured, via, for instance, optical scanning of the paper or other medium, then compiled, interpreted or otherwise processed in a suitable manner if necessary, and then stored in a computer memory.

[0183] Computer program code for carrying out operations of the present invention may be written in an object oriented programming language such as Java7, Smalltalk, Python, Labview, C++, or VisualBasic. However, the computer program code for carrying out operations of the present invention may also be written in conventional procedural programming languages, such as the "C" programming language or even assembly language. The program code may execute entirely on the user's computer, partly on the user's computer, as a stand-alone software package, partly on the user's computer and partly on a remote computer or entirely on the remote computer. In the latter scenario, the remote computer may be connected to the user's computer through a local area network (LAN) or a wide area network (WAN) or secured area network (SAN), or the connection may be made to an external computer (for example, through the Internet using an Internet Service Provider).

[0184] The flowcharts and block diagrams of certain of the figures herein illustrate the architecture, functionality, and operation of possible implementations of analysis models and evaluation systems and/or programs according to the present invention. In this regard, each block in the flow charts or block diagrams represents a module, segment, operation, or portion of code, which comprises one or more executable instructions for implementing the specified logical function(s). It should also be noted that in some alternative implementations, the functions noted in the blocks might occur out of the order noted in the figures. For example, two blocks shown in succession may in fact be executed substantially concurrently or the blocks may sometimes be executed in the reverse order, depending upon the functionality involved.

[0185] **Figure 23** is a block diagram of exemplary embodiments of data processing systems **305** that illustrates systems, methods, and computer program products in accordance with embodiments of the present invention. The processor **310** communicates with the memory **314** via an address/data bus **348.** The processor **310** can be any commercially available or custom microprocessor. The memory **314** is representative of the overall hierarchy of memory devices containing the software and data used to implement the functionality of the data processing system **305.** The memory **314** can include, but is not limited to, the following types of devices: cache, ROM, PROM, EPROM, EEPROM, flash memory, SRAM, and DRAM.

[0186] As shown in **Figure 23,** the memory **314** may include several categories of software and data used in the data processing system **305:** the operating system **352**; the application programs **354**; the input/output (I/O) device drivers **358**; an STR, IR and/or DRI risk score module **370** and the data **356.** The Module **370** can consider the level of defined metabolite and lipoprotein parameters of the defined multi-marker STR and IR parameters which can include a measurement of GlycA, lipoprotein components and Valine and also optionally, glucose, in a multi-parameter mathematical model of risk of developing type 2 diabetes in a defined timeline, e.g.., the next 5 years or a likelihood of having prediabetes.

[0187] The data **356** may include signal (constituent and/or composite spectrum lineshape) or measurement data of the lipoproteins and metabolite parameters **362** which may be obtained from a data or signal acquisition system **320.**

As will be appreciated by those of skill in the art, the operating system **352** may be any operating system suitable for use with a data processing system, such as OS/2, AIX or OS/390 from International Business Machines Corporation, Armonk, NY, WindowsCE, WindowsNT, Windows95, Windows98, Windows2000 or WindowsXP from Microsoft Corporation, Redmond, WA, PalmOS from Palm, Inc., MacOS from Apple Computer, UNIX, FreeBSD, or Linux, proprietary operating systems or dedicated operating systems, for example, for embedded data processing systems.

[0188] The I/O device drivers **358** typically include software routines accessed through the operating system **352** by the application programs **354** to communicate with devices such as I/O data port(s), data storage **356** and certain memory **314** components and/or the analyzer **22.** The application programs **354** are illustrative of the programs that implement the various features of the data processing system **305** and can include at least one application, which supports operations according to embodiments of the present invention. Finally, the data **356** represents the static and dynamic data used by the application programs **354,** the operating system **352,** the I/O device drivers **358,** and other software programs that may reside in the memory **314.**

[0189] While the present invention is illustrated, for example, with reference to the Module **370** being an application program in **Figure 23,** as will be appreciated by those of skill in the art, other configurations may also be utilized while still benefiting from the teachings of the present invention. For example, the Module **370** may also be incorporated into the operating system **352,** the I/O device drivers **358** or other such logical division of the data processing system **305.** Thus, the present invention should not be construed as limited to the configuration of **Figure 23,** which is intended to encompass any configuration capable of carrying out the operations described herein.

[0190] **Figure 24** is a flow chart of exemplary operations that can carry out embodiments of the present invention. In some embodiments, the inflammatory marker is an NMR derived GlycA which can employ actions associated with blocks **500, 502, 515, 520** and optionally **503, 412** and **522.** However, where other inflammatory markers are used, the methods can include only blocks, **423, 524, 525** and **526,** according to some embodiments.

[0191] If GlycA is the inflammatory marker, the method can include obtaining a (measured) composite envelope NMR spectrum of NMR spectra of a fitting region of a biosample (e.g., blood plasma or serum) can be obtained (block **500**). The NMR composite signal envelope is electronically deconvolved using a defined model having HDL, LDL and VLDL/Chylos components and a plurality of curve fit (e.g., Lorentzian) functions associated with at least a GlycA peak region centered at a defined chemical shift location (e.g., 2.00 ppm) associated with GlycA (block **502**). A defined number of curve fitting functions for the peak region associated with GlycA can be summed (block **515**). A conversion factor can be applied to the summed functions to generate a calculated measurement of GlycA (block **520**).

[0192] The method can include providing a STR risk factor score to identify potential β-cell dysfunction and/or impairment or a change in β-cell status (block **523**). The change in status can be an improvement or further impairment associated with a therapy, such as a drug therapy, for example.

[0193] The method can include providing an IR risk score associated with insulin resistance and the risk of conversion or progression to type 2 diabetes, typically within 5-7 years (block **524**).

[0194] The method can include combining the IR and STR scores (block **525**) and identifying whether the patient is at risk of developing type 2 diabetes and/or has prediabetes based on the combined IR and STR scores as a DRI score (block **526**). The IR score may be weighted to have an increased value relative to its un-weighted score to provide a greater input into the DRI score relative to the STR score or may naturally have a more dominant score in longer term evaluations.

[0195] The STR and IR scores can be generated using defined models of risk with associated defined coefficients for defined lipoprotein and metabolite parameters to generate STR and IR scores that generate a DRI score in a range between 1-10.

[0196] Optionally, the DRI and/or GlycA scores can be provided in a patient and/or clinical trial report (block **522**).

[0197] The defined GlycA deconvolution model can include a protein signal component at a density greater than about 1.21g/L that can be deconvolved/ separated from the signal composite envelope (block **503**).

[0198] The foregoing is illustrative of the present invention and is not to be construed as limiting thereof. Although a few exemplary embodiments of this invention have been described, those skilled in the art will readily appreciate that many modifications are possible in the exemplary embodiments without materially departing from the novel teachings and advantages of this invention. Accordingly, all such modifications are intended to be included within the scope of this invention as defined in the claims. In the claims, means-plus-function clauses, where used, are intended to cover the structures described herein as performing the recited function and not only structural equivalents but also equivalent structures. Therefore, it is to be understood that the foregoing is illustrative of the present invention and is not to be construed as limited to the specific embodiments disclosed, and that modifications to the disclosed embodiments, as well as other embodiments, are intended to be included within the scope of the appended claims.

Claims

1. A computer-implemented method of evaluating a subject's risk of developing type 2 diabetes, comprising:
calculating a short term diabetes risk factor (SDRF) score of a subject using a defined mathematical model of risk of developing type 2 diabetes, wherein the defined mathematical model includes a concentration measurement of a defined high density lipoprotein particle (HDL-P) subpopulation, a concentration measurement of at least one inflammatory marker and a concentration measurement of at least one interaction parameter as components of the model which are mathematically combined with respective defined coefficients to generate the calculated SDRF score, wherein the SDRF score is calculated using the following equation:

$$\text{SDRF score} = -(A)(\text{HDL-P}_{\text{MED}}) - (B)(\text{GlycA}) + (C)(\text{GlycA} \times \text{HDL-P}_{\text{MED}}),$$

wherein A, B and C are defined beta coefficients from a logistic regression model for short term conversion to diabetes as the defined mathematical model of risk of developing type 2 diabetes, and wherein GlycA is the inflammatory marker, $\text{HDL-P}_{\text{MED}}$ is a medium size HDL-P subpopulation, and the interaction parameter is GlycA multiplied by the concentration of $\text{HDL-P}_{\text{MED}}$, and wherein the subject is at risk of converting to type 2 diabetes mellitus within 5 years when the SDRF score is at a third tertile value or greater value of a population norm.

2. The method of Claim 1, wherein the particle diameters of the said $\text{HDL-P}_{\text{MED}}$ subpopulation are between 8.3 nm and one of 9.4 nm, 10.0 nm and 10.2 nm.

3. The method of Claim 1 or Claim 2, further comprising calculating an insulin resistance (IR) score of the subject using a defined mathematical model of insulin resistance, this model optionally including a plurality of components including a concentration measurement of a defined HDL-P subpopulation, which may be the concentration measurement of the defined HDL-P subpopulation used to calculate the SDRF score, the measurement of the inflammatory marker, a measurement of a defined subpopulation of VLDL-P (very low density lipoprotein /chylomicron particle subclasses), an IR index with a range of between 0-100, the range representing from low to high, insulin sensitivity to insulin resistance, and a measurement of a branched chain amino acid (BCAA), obtained from the at least one *in vitro* biosample of the subject, optionally wherein the components of the defined mathematical model of IR are mathematically combined to generate the IR score.

4. The method of Claim 3, wherein the measurement of the defined sub-population of VLDL-P is a concentration of medium VLDL-P, and wherein the BCAA is Valine.

5. The method of Claim 3, further comprising calculating a diabetes risk score by combining the SDRF score and the IR score, optionally wherein coefficients of the SDRF and IR scores are derived from a logistic regression model that includes SDRF and IR to predict actual 5 year conversion to diabetes using at least one defined population study to thereby generate a DRI score with a risk of conversion within 5 years irrespective of a glucose value of the subject.

6. The method of any preceding Claim, further comprising evaluating a measured glucose and/or HbA1c value of the subject, and electronically providing a report with a 5 year risk of conversion to Type 2 diabetes risk estimate based on the glucose measurement and the DRI score.

7. The method of any of Claims 3-5, or claim 6 when dependent on claim 3, wherein the IR score is an eLP-IR score and is calculated using the following equation:

$$\text{eLP-IR} = (A)(\text{LP-IR}) + (B)(\text{Valine}) - (C)(\text{VLDL-P}_{\text{MED}}) - (D)(\text{HDL-P}_{\text{MED}}) + (E)(\text{GlycA}),$$

wherein A, B, C, D and E are defined beta coefficients from a linear regression model for insulin resistance, $\text{VLDL-P}_{\text{MED}}$ is a concentration of a medium VLDL-P subpopulation, valine is a branched chain amino acid, and LP-IR is a lipoprotein insulin resistance index calculated using six defined lipoprotein subclasses and has a numerical value in a range of 0-100 representing insulin sensitive to insulin resistance.

8. The method of Claim 1, further comprising at least one of: evaluating a drug therapy, evaluating a clinical trial, or evaluating candidates for drug discovery, using the SDRF score, and optionally further comprising calculating a

plurality of the SDRF scores over time from respective biosamples to thereby evaluate a change in SDRF score to conversion to type-2 diabetes.

9. The method of Claim 1, wherein raw scores associated with the SDRF score are between -6.4 and -1.6, wherein <-4.1 is associated with about a 25th percentile and >-3.8 is associated with about a 75th percentile of the study population, and wherein ≥-3.8 values indicate an increased risk of early conversion to type 2 diabetes independent of glycemic value that can stratify risk of conversion to type 2 diabetes for subjects having a common glycemic measurement with different SDRF scores.

10. The method of Claim 1, wherein the SDRF score is provided in a report in a defined numerical score range, with scores associated with a fourth quartile (4Q), fifth quintile (5Q) or 10^{th} decile of a population norm reflecting an increased risk of developing type 2 diabetes within 2 years relative to lower scores.

11. The method of any of Claims 3-5 or 7, or Claim 6 when dependent on Claim 3, further comprising generating a DRI score using the following equation:

$$\text{DRI score} = X(\text{IR score}) + Y(\text{SDRF}),$$

wherein X and Y are coefficients defined by a logistic regression model for 5-year diabetes conversion in people with glucose <110 mg/dL using a defined study population, and wherein the DRI score is mathematically altered into a DRI score range using a plurality of equal subparts over a range of possible DRI raw scores.

12. A computer-implemented method of identifying subjects that are likely to benefit from a drug therapy such as reconstituted HDL infusion to inhibit Type 2 diabetes mellitus (T2DM), comprising:

generating a defined short term diabetes risk factor (SDRF) score using measurements of defined lipoprotein and metabolite components of a biosample of a subject, wherein the components include a high density lipoprotein particle (HDL-P) subpopulation and an interaction parameter using the HDL-P subpopulation and an inflammatory marker, wherein the SDRF score is calculated using the following equation:

$$\text{SDRF score} = -(A)(\text{HDL-P}_{\text{MED}}) - (B)(\text{GlycA}) + (C)(\text{GlycA x HDL-P}_{\text{MED}}),$$

wherein A, B and C are defined beta coefficients from a logistic regression model for short term conversion to diabetes as the defined mathematical model of risk of developing type 2 diabetes, and wherein GlycA is the inflammatory marker, $\text{HDL-P}_{\text{MED}}$ is a medium size HDL-P subpopulation, and GlycA x $\text{HDL-P}_{\text{MED}}$ is the interaction parameter; and
identifying subjects that have an increased SDRF score relative to a defined population norm which indicates that the subject is likely to benefit from therapy to inhibit T2DM.

13. The method of any preceding Claim, wherein the measurements of the biosample for the SDRF score are NMR-derived measurements.

Patentansprüche

1. Ein computerimplementiertes Verfahren zur Evaluierung des Risikos eines Individuums für die Entstehung von Typ-2-Diabetes, das Folgendes beinhaltet:
Berechnen eines Kurzzeit-Diabetesrisikofaktor(SDRF)-Scores eines Individuums unter Verwendung eines definierten mathematischen Modells für das Risiko für die Entstehung von Typ-2-Diabetes, wobei das definierte mathematische Modell eine Konzentrationsmessung einer definierten Subpopulation der Partikel von Lipoprotein hoher Dichte (HDL-P), eine Konzentrationsmessung von mindestens einem Entzündungsmarker und eine Konzentrationsmessung von mindestens einem Interaktionsparameter als Komponenten des Modells umfasst, die mathematisch mit den jeweiligen definierten Koeffizienten kombiniert werden, um den berechneten SDRF-Score zu erzeugen, wobei der SDRF-Score unter Verwendung der folgenden Gleichung berechnet wird:

$$\text{SDRF-Score} = -(A)(\text{HDL-P}_{\text{MED}}) - (B)(\text{GlycA}) + (C)(\text{GlycA x HDL-P}_{\text{MED}}),$$

wobei A, B und C definierte Beta-Koeffizienten aus einem logistischen Regressionsmodell für die Kurzzeit-Konversion zu Diabetes als das definierte mathematische Modell des Risikos für die Entstehung von Typ-2-Diabetes sind und wobei GlycA der Entzündungsmarker ist, HDL-P_{MED} eine HDL-P-Subpopulation mittlerer Größe ist und der Interaktionsparameter GlycA, multipliziert mit der Konzentration von HDL-P_{MED}, ist und wobei bei dem Individuum ein Risiko für die Konversion zu Typ-2-Diabetes mellitus innerhalb von 5 Jahren besteht, wenn der SDRF-Score ein im dritten Tertil einer Populationsnorm liegender Wert oder ein höherer Wert ist.

2. Verfahren gemäß Anspruch 1, wobei die Partikeldurchmesser der HDL-P_{MED}-Subpopulation zwischen 8,3 nm und einem von 9,4 nm, 10,0 nm und 10,2 nm betragen.

3. Verfahren gemäß Anspruch 1 oder Anspruch 2, das ferner das Berechnen eines Insulinresistenz(IR)-Scores des Individuums unter Verwendung eines definierten mathematischen Modells der Insulinresistenz beinhaltet, wobei dieses Modell optional eine Vielzahl von Komponenten umfasst, die Folgendes umfassen: eine Konzentrationsmessung einer definierten HDL-P-Subpopulation, welche die zum Berechnen des SDRF-Score verwendete Konzentrationsmessung der definierten HDL-P-Subpopulation sein kann, die Messung des Entzündungsmarkers, eine Messung einer definierten Subpopulation von VLDL-P (Lipoprotein sehr niedriger Dichte/Chylomikronpartikel-Unterklassen), ein IR-Index mit einem Bereich zwischen 0-100, wobei der Bereich eine niedrige bis hohe Insulinempfindlichkeit bis hin zu Insulinresistenz darstellt, und eine Messung einer verzweigtkettigen Aminosäure (BCAA), die aus der mindestens einen biologischen In-vitro-Probe des Individuums erhalten wird, wobei die Komponenten des definierten mathematischen Modells der IR optional mathematisch kombiniert werden, um den IR-Score zu erzeugen.

4. Verfahren gemäß Anspruch 3, wobei die Messung der definierten Subpopulation von VLDL-P eine Konzentration von mittlerem VLDL-P ist und wobei die BCAA Valin ist.

5. Verfahren gemäß Anspruch 3, das ferner das Berechnen eines Diabetesrisiko-Scores durch Kombinieren des SDRF-Scores und des IR-Scores beinhaltet, wobei Koeffizienten der SDRF- und IR-Scores optional aus einem logistischen Regressionsmodell, das SDRF und IR umfasst, zur Vorhersage der tatsächlichen 5-Jahres-Konversion zu Diabetes abgeleitet sind, wobei mindestens eine definierte Populationsstudie verwendet wird, um dadurch einen DRI-Score mit einem Risiko für eine Konversion innerhalb von 5 Jahren, unabhängig von einem Glucosewert des Individuums, zu erzeugen.

6. Verfahren gemäß einem vorhergehenden Anspruch, das ferner das Evaluieren eines gemessenen Glucose- und/oder HbAlc-Werts des Individuums und das elektronische Bereitstellen eines Berichts mit einer Risikoabschätzung des 5-Jahres-Risikos für eine Konversion zu Typ-2-Diabetes auf der Grundlage der Glucosemessung und des DRI-Scores beinhaltet.

7. Verfahren nach einem der Ansprüche 3 bis 5 oder Anspruch 6 bei Abhängigkeit von Anspruch 3, wobei der IR-Score ein eLP-IR-Score ist und unter Verwendung der folgenden Gleichung berechnet wird:

$$\text{eLP-IR} = (A)(\text{LP-IR}) + (B)(\text{Valin}) - (C)(\text{VLDL-PMED}) - (D)(\text{HDL-P}_{\text{MED}}) + (E)(\text{GlycA}),$$

wobei A, B, C, D und E definierte Beta-Koeffizienten aus einem linearen Regressionsmodell für Insulinresistenz sind, VLDL-P_{MED} eine Konzentration einer mittleren VLDL-P-Subpopulation ist, Valin eine verzweigtkettige Aminosäure ist und LP-IR ein Lipoprotein-Insulinresistenzindex ist, der unter Verwendung von sechs definierten Lipoproteinunterklassen berechnet wird und einen numerischen Wert in einem Bereich von 0-100 aufweist, der Insulinempfindlichkeit bis hin zur Insulinresistenz darstellt.

8. Verfahren gemäß Anspruch 1, das ferner mindestens eines der Folgenden beinhaltet: Evaluieren einer Arzneimitteltherapie, Evaluieren einer klinischen Prüfung oder Evaluieren von Arzneimittelentdeckungskandidaten, unter Verwendung des SDRF-Scores, und das optional ferner das Berechnen einer Vielzahl der SDRF-Scores im Zeitverlauf aus den jeweiligen biologischen Proben beinhaltet, um dadurch eine Änderung des SDRF-Scores hin zu einer Konversion zu Typ-2-Diabetes zu evaluieren.

9. Verfahren gemäß Anspruch 1, wobei die mit dem SDRF-Score assoziierten Roh-Scores zwischen -6,4 und -1,6 betragen, wobei < -4,1 mit etwa einem 25. Perzentil assoziiert ist und > -3,8 mit etwa einem 75. Perzentil der Studienpopulation assoziiert ist, und wobei Werte ≥ -3,8 ein erhöhtes Risiko für eine frühe Konversion zu Typ-2-Diabetes unabhängig von dem glykämischen Wert anzeigen, wodurch das Risiko für eine Konversion zu Typ-2-Diabetes für Individuen, die eine gemeinsame glykämische Messung bei unterschiedlichen SDRF-Scores aufweisen, stratifiziert werden kann.

10. Verfahren gemäß Anspruch 1, wobei der SDRF-Score in einem Bericht in einem definierten numerischen Score-Bereich bereitgestellt wird, wobei die Scores, die mit einem vierten Quartil (4Q), fünften Quintil (5Q) oder 10. Dezil einer Populationsnorm assoziiert sind, ein erhöhtes Risiko für die Entstehung von Typ-2-Diabetes innerhalb von 2 Jahren gegenüber niedrigeren Scores widerspiegeln.

11. Verfahren gemäß einem der Ansprüche 3 bis 5 oder 7, oder Anspruch 6 bei Abhängigkeit von Anspruch 3, das ferner das Erzeugen eines DRI-Scores unter Verwendung der folgenden Gleichung beinhaltet:

$$\text{DRI-Score} = X(\text{IR-Score}) + Y(\text{SDRF}),$$

wobei X und Y durch ein logistisches Regressionsmodell definierte Koeffizienten für die 5-Jahres-Diabeteskonversion bei Menschen mit Glucose < 110 mg/dl unter Verwendung einer definierten Studienpopulation sind und wobei der DRI-Score unter Verwendung einer Vielzahl von gleichen Unterabschnitten über einen Bereich möglicher DRI-Roh-Scores mathematisch in einen DRI-Score-Bereich abgeändert wird.

12. Ein computerimplementiertes Verfahren zum Identifizieren von Individuen, die wahrscheinlich einen Nutzen aus einer Arzneimitteltherapie, wie etwa einer rekonstituierten HDL-Infusion zum Hemmen von Typ-2-Diabetes mellitus (T2DM) ziehen werden, das Folgendes beinhaltet:

Erzeugen eines definierten Kurzzeit-Diabetesrisikofaktor(SDRF)-Scores unter Verwendung von Messungen definierter Lipoprotein- und Metaboliten-Komponenten einer biologischen Probe eines Individuums, wobei die Komponenten eine Subpopulation der Partikel von Lipoprotein hoher Dichte (HDL-P) und einen Interaktionsparameter unter Verwendung der HDL-P-Subpopulation und einen Entzündungsmarker beinhalten, wobei der SDRF-Score unter Verwendung der folgenden Gleichung berechnet wird:

$$\text{SDRF-Score} = -(A)(\text{HDL-P}_{\text{MED}}) - (B)(\text{GlycA}) + (C)(\text{GlycA x HDL-P}_{\text{MED}}),$$

wobei A, B und C definierte Beta-Koeffizienten aus einem logistischen Regressionsmodell für die Kurzzeit-Konversion zu Diabetes als definiertes mathematisches Modell des Risikos für die Entstehung von Typ-2-Diabetes sind und wobei GlycA der Entzündungsmarker ist, $\text{HDL-P}_{\text{MED}}$ eine HDL-P-Subpopulation mittlerer Größe ist und GlycA x $\text{HDL-P}_{\text{MED}}$ der Interaktionsparameter ist; und
Identifizieren von Individuen, die einen SDRF-Score gegenüber einer definierten Populationsnorm aufweisen, der anzeigt, dass das Individuum wahrscheinlich einen Nutzen aus der Therapie zum Hemmen von T2DM ziehen wird.

13. Verfahren gemäß einem vorhergehenden Anspruch, wobei die Messungen der biologischen Probe für den SDRF-Score von NMR abgeleitete Messungen sind.

Revendications

1. Méthode mise en œuvre par ordinateur pour évaluer le risque de développement d'un diabète de type 2 chez un sujet, comprenant :
le calcul d'un score du facteur de risque de diabète à court terme (SDRF) d'un sujet en utilisant un modèle mathématique défini du risque de développement d'un diabète de type 2, où le modèle mathématique défini inclut une mesure de la concentration en une sous-population définie de particules de lipoprotéines de haute densité (HDL-P), une mesure de la concentration en au moins un marqueur inflammatoire et une mesure de la concentration en au moins un paramètre d'interaction en tant que composants du modèle qui sont mathématiquement combinés à des coefficients définis respectifs pour générer le score du SDRF calculé, le score du SDRF étant calculé au moyen

de l'équation suivante :

$$\text{Score du SDRF} = -(A)\,(\text{HDL-P}_{\text{MED}}) - (B)\,(\text{GlycA}) + (C)\,(\text{GlycA x HDL-P}_{\text{MED}}),$$

où A, B et C sont des coefficients bêta définis d'un modèle de régression logistique de la conversion au diabète à court terme en tant que modèle mathématique défini du risque de développement d'un diabète de type 2 et où GlycA est le marqueur inflammatoire, HDL-P_{MED} est une sous-population d'HDL-P de tailles moyennes et le paramètre d'interaction est GlycA multiplié par la concentration en HDL-P_{MED}, et où le sujet est à risque de conversion au diabète sucré de type 2 en l'espace de 5 ans si la valeur du score du SDRF se situe dans le troisième tertile ou dans la plage la plus élevée d'une norme de population.

2. Méthode de la revendication 1, où les diamètres des particules de ladite sous-population d'HDL-P_{MED} sont entre 8,3 nm et un parmi 9,4 nm, 10,0 nm et 10,2 nm.

3. Méthode de la revendication 1 ou de la revendication 2 comprenant en outre le calcul d'un score d'insulinorésistance (IR) du sujet en utilisant un modèle mathématique défini de l'insulinorésistance, ce modèle incluant éventuellement une pluralité de composants, y compris une mesure de la concentration en une sous-population définie d'HDL-P qui peut être la mesure de la concentration en la sous-population définie d'HDL-P utilisée pour calculer le score du SDRF, la mesure du marqueur inflammatoire, une mesure d'une sous-population définie de VLDL-P (sous-classes des particules de lipoprotéines de très basse densité/chylomicrons), un indice d'IR dans une plage entre 0-100, la plage allant, de la limite basse à la limite élevée, d'une insulinosensibilité à une insulinorésistance, et une mesure d'un acide aminé à chaîne ramifiée (BCAA), obtenues à partir du au moins un échantillon biologique *in vitro* du sujet, éventuellement où les composants du modèle mathématique défini d'IR sont mathématiquement combinés pour générer le score d'IR.

4. Méthode de la revendication 3, où la mesure de la sous-population définie de VLDL-P est une concentration en VLDL-P de tailles moyennes et où le BCAA est la valine.

5. Méthode de la revendication 3 comprenant en outre le calcul d'un score du risque de diabète par combinaison du score du SDRF et du score d'IR, éventuellement où les coefficients des scores du SDRF et d'IR sont dérivés d'un modèle de régression logistique qui inclut le SDRF et l'IR pour prédire la conversion actuelle au diabète à 5 ans en utilisant au moins une étude de population définie pour ainsi générer un score de l'indice de risque de diabète (DRI) associé à un risque de conversion en l'espace de 5 ans, indépendamment d'une valeur de la glycémie du sujet.

6. Méthode de l'une quelconque des revendications précédentes comprenant en outre une évaluation d'une valeur de la glycémie et/ou de l'HbA1c mesurée chez le sujet et la production électronique d'un rapport de l'estimation du risque de conversion au diabète de type 2 à 5 ans sur la base de la mesure de la glycémie et du score de DRI.

7. Méthode de l'une quelconque des revendications 3-5 ou de la revendication 6 placée sous la dépendance de la revendication 3, où le score d'IR est un score d'eLP-IR calculé au moyen de l'équation suivante :

$$\text{eLP-IR} = (A)\,(\text{LP-IR}) + (B)\,(\text{Valine}) - (C)\,(\text{VLDL-P}_{\text{MED}}) - (D)\,(\text{HDL-P}_{\text{MED}}) + (E)\,(\text{GlycA}),$$

où A, B, C, D et E sont des coefficients bêta définis d'un modèle de régression linéaire de l'insulinorésistance, VLDL-P_{MED} est une concentration en une sous-population de VLDL-P de tailles moyennes, valine est un acide aminé à chaîne ramifiée et LP-IR est un indice d'insulinorésistance associé aux taux de lipoprotéines calculé en utilisant six sous-classes définies de lipoprotéines et dont la valeur numérique est dans une plage de 0-100, 0 représentant une insulinosensibilité et 100 une insulinorésistance.

8. Méthode de la revendication 1 comprenant en outre au moins un des éléments suivants : évaluation d'un traitement médicamenteux, évaluation d'un essai clinique ou évaluation de candidats dans le cadre de la découverte de médicaments en utilisant le score du SDRF, et comprenant en outre éventuellement le calcul d'une pluralité de scores du SDRF en fonction du temps à partir d'échantillons biologiques respectifs pour ainsi évaluer un changement du score du SDRF indicatif d'une conversion au diabète de type 2.

9. Méthode de la revendication 1, où les scores bruts associés au score du SDRF sont entre -6,4 et -1,6, où <-4,1 est associé à un 25ème percentile environ et >-3,8 est associé à un 75ème percentile environ de la population à l'étude, et où des valeurs ≥-3,8 sont indicatrices d'un risque accru de conversion précoce au diabète de type 2, indépendamment de la valeur de la glycémie, qui peut permettre de stratifier la conversion au diabète de type 2 chez des sujets chez lesquels des mesures de la glycémie sont les mêmes mais les scores du SDRF différents.

10. Méthode de la revendication 1, où le score du SDRF est fourni dans un rapport sous la forme d'une plage définie de scores numériques, des scores associés à un quatrième quartile (4Q), un cinquième quintile (5Q) ou un 10ème décile d'une norme de population réfléchissant un risque accru de développement d'un diabète de type 2 en l'espace de 2 ans par comparaison à des scores plus faibles.

11. Méthode de l'une quelconque des revendications 3-5 ou 7 ou de la revendication 6 placée sous la dépendance de la revendication 3, comprenant en outre la génération d'un score de DRI au moyen de l'équation suivante :

$$\text{Score de DRI} = X\ (\text{score d'IR}) + Y\ (\text{SDRF}),$$

où X et Y sont des coefficients définis d'un modèle de régression logistique de la conversion au diabète à 5 ans chez des sujets ayant une glycémie <110 mg/dl en utilisant une population à l'étude définie, et où le score de DRI est mathématiquement transformé en une plage de scores de DRI en utilisant une pluralité de sous-parties égales sur une plage de scores de DRI bruts possibles.

12. Méthode mise en œuvre par ordinateur pour identifier des sujets susceptibles de bénéficier d'un traitement médicamenteux tel que la perfusion d'HDL reconstituées pour inhiber le diabète sucré de type 2 (T2DM), comprenant :

la génération d'un score défini du facteur de risque de diabète à court terme (SDRF) en utilisant des mesures de composants lipoprotéiniques et métaboliques définis obtenues avec un échantillon biologique d'un sujet, où les composants incluent une sous-population de particules de lipoprotéines de haute densité (HDL-P) et un paramètre d'interaction utilisant la sous-population d'HDL-P et un marqueur inflammatoire, le score du SDRF étant calculé au moyen de l'équation suivante :

$$\text{Score du SDRF} = -(A)\ (\text{HDL-P}_{\text{MED}}) - (B)\ (\text{GlycA}) + (C)\ (\text{GlycA x HDL-P}_{\text{MED}}),$$

où A, B et C sont des coefficients bêta définis d'un modèle de régression logistique de la conversion au diabète à court terme en tant que modèle mathématique défini du risque de développement d'un diabète de type 2 et où GlycA est le marqueur inflammatoire, $\text{HDL-P}_{\text{MED}}$ est une sous-population d'HDL-P de tailles moyennes et $\text{GlycA x HDL-P}_{\text{MED}}$ est le paramètre d'interaction ; et
l'identification de sujets chez lesquels le score du SDRF est accru par comparaison à une norme de population définie, indiquant que le sujet est susceptible de bénéficier d'un traitement pour inhiber un T2DM.

13. Méthode de l'une quelconque des revendications précédentes, où les mesures obtenues avec l'échantillon biologique pour déterminer le score du SDRF sont des mesures dérivant de la RMN

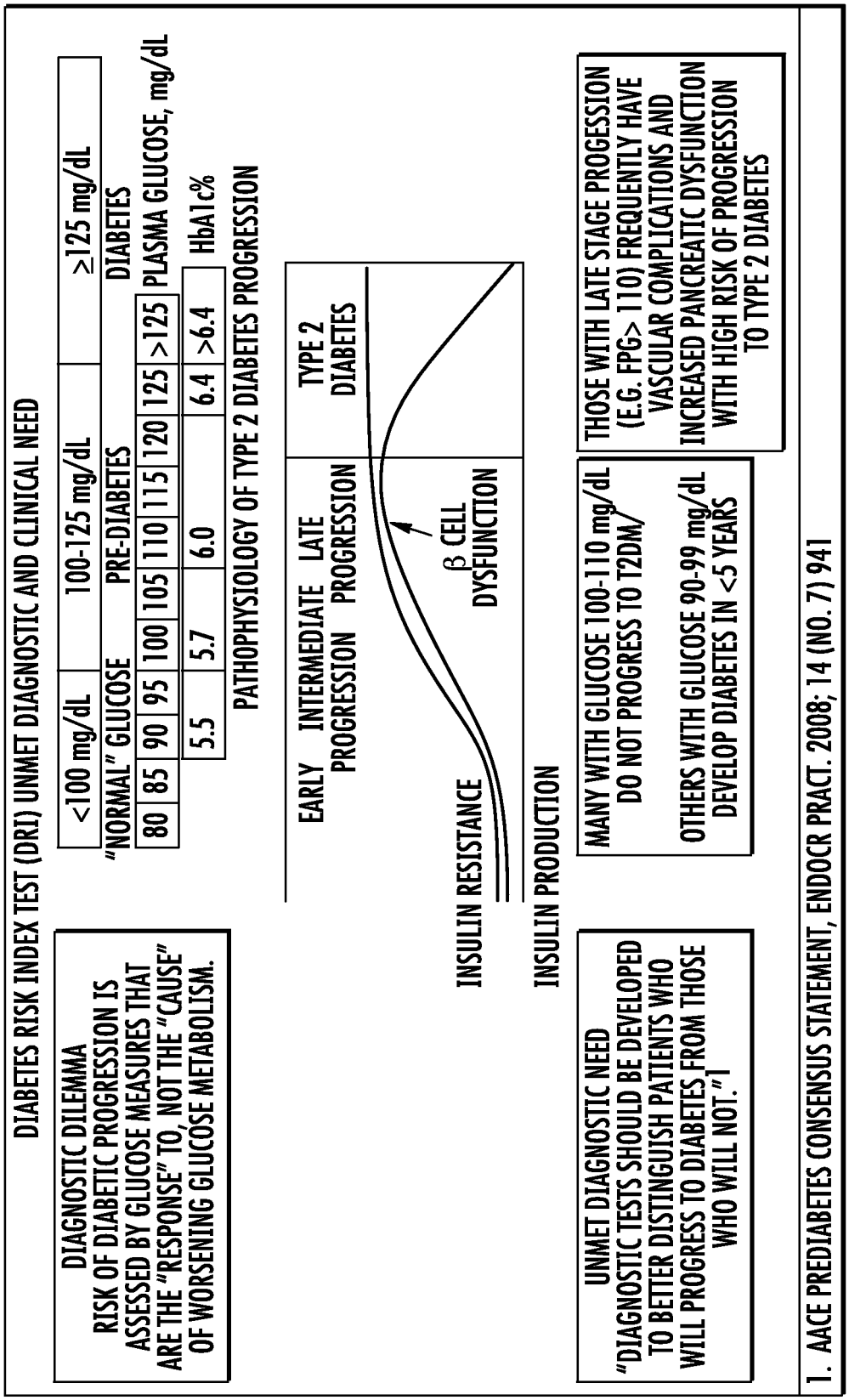
DIABETES RISK INDEX TEST (DRI) UNMET DIAGNOSTIC AND CLINICAL NEED
DIAGNOSTIC DILEMMA
RISK OF DIABETIC PROGRESSION IS ASSESSED BY GLUCOSE MEASURES THAT ARE THE "RESPONSE" TO, NOT THE "CAUSE" OF WORSENING GLUCOSE METABOLISM.
<100 mg/dL
100-125 mg/dL
≥125 mg/dL
"NORMAL" GLUCOSE
PRE-DIABETES
DIABETES
80 85 90 95 100 105 110 115 120 125 >125 PLASMA GLUCOSE, mg/dL
5.5 5.7 6.0 6.4 >6.4 HbA1c%
PATHOPHYSIOLOGY OF TYPE 2 DIABETES PROGRESSION
EARLY INTERMEDIATE LATE PROGRESSION PROGRESSION
TYPE 2 DIABETES
INSULIN RESISTANCE
β CELL DYSFUNCTION
INSULIN PRODUCTION
UNMET DIAGNOSTIC NEED
"DIAGNOSTIC TESTS SHOULD BE DEVELOPED TO BETTER DISTINGUISH PATIENTS WHO WILL PROGRESS TO DIABETES FROM THOSE WHO WILL NOT."[1]
MANY WITH GLUCOSE 100-110 mg/dL DO NOT PROGRESS TO T2DM/
OTHERS WITH GLUCOSE 90-99 mg/dL DEVELOP DIABETES IN <5 YEARS
THOSE WITH LATE STAGE PROGESSION (E.G. FPG> 110) FREQUENTLY HAVE VASCULAR COMPLICATIONS AND INCREASED PANCREATIC DYSFUNCTION WITH HIGH RISK OF PROGRESSION TO TYPE 2 DIABETES
1. AACE PREDIABETES CONSENSUS STATEMENT, ENDOCR PRACT. 2008; 14 (NO. 7) 941

FIG. 1A

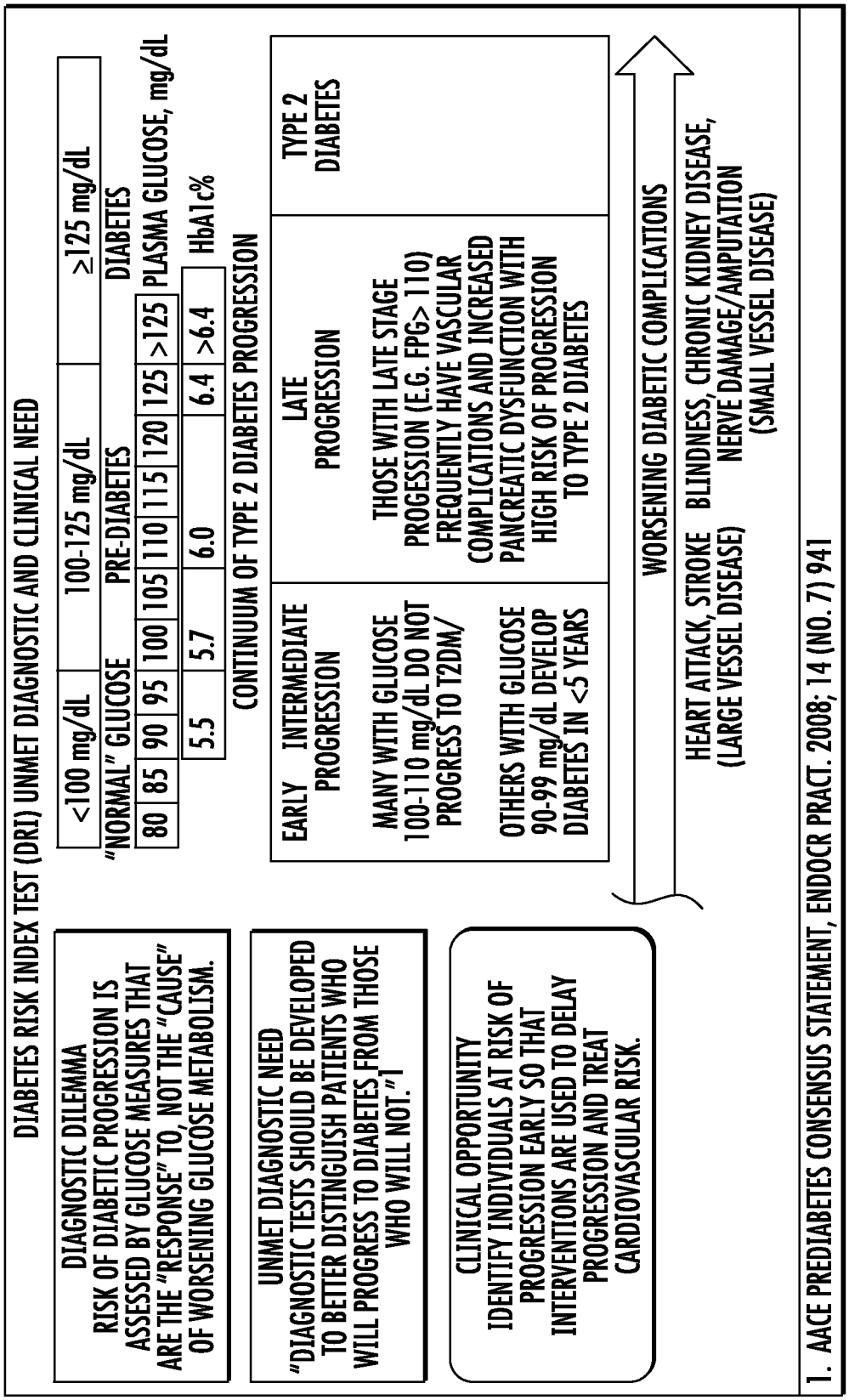
DIABETES RISK INDEX TEST (DRI) UNMET DIAGNOSTIC AND CLINICAL NEED
DIAGNOSTIC DILEMMA
RISK OF DIABETIC PROGRESSION IS ASSESSED BY GLUCOSE MEASURES THAT ARE THE "RESPONSE" TO, NOT THE "CAUSE" OF WORSENING GLUCOSE METABOLISM.
UNMET DIAGNOSTIC NEED
"DIAGNOSTIC TESTS SHOULD BE DEVELOPED TO BETTER DISTINGUISH PATIENTS WHO WILL PROGRESS TO DIABETES FROM THOSE WHO WILL NOT."[1]
CLINICAL OPPORTUNITY
IDENTIFY INDIVIDUALS AT RISK OF PROGRESSION EARLY SO THAT INTERVENTIONS ARE USED TO DELAY PROGRESSION AND TREAT CARDIOVASCULAR RISK.
<100 mg/dL
100-125 mg/dL
≥125 mg/dL
"NORMAL" GLUCOSE
PRE-DIABETES
DIABETES
80 85 90 95 100 105 110 115 120 125 >125 PLASMA GLUCOSE, mg/dL
5.5 5.7 6.0 6.4 >6.4 HbA1c%
CONTINUUM OF TYPE 2 DIABETES PROGRESSION
EARLY INTERMEDIATE PROGRESSION
MANY WITH GLUCOSE 100-110 mg/dL DO NOT PROGRESS TO T2DM/
OTHERS WITH GLUCOSE 90-99 mg/dL DEVELOP DIABETES IN <5 YEARS
LATE PROGRESSION
THOSE WITH LATE STAGE PROGESSION (E.G. FPG> 110) FREQUENTLY HAVE VASCULAR COMPLICATIONS AND INCREASED PANCREATIC DYSFUNCTION WITH HIGH RISK OF PROGRESSION TO TYPE 2 DIABETES
TYPE 2 DIABETES
WORSENING DIABETIC COMPLICATIONS
HEART ATTACK, STROKE (LARGE VESSEL DISEASE)
BLINDNESS, CHRONIC KIDNEY DISEASE, NERVE DAMAGE/AMPUTATION (SMALL VESSEL DISEASE)
1. AACE PREDIABETES CONSENSUS STATEMENT, ENDOCR PRACT. 2008; 14 (NO. 7) 941

FIG. 1B

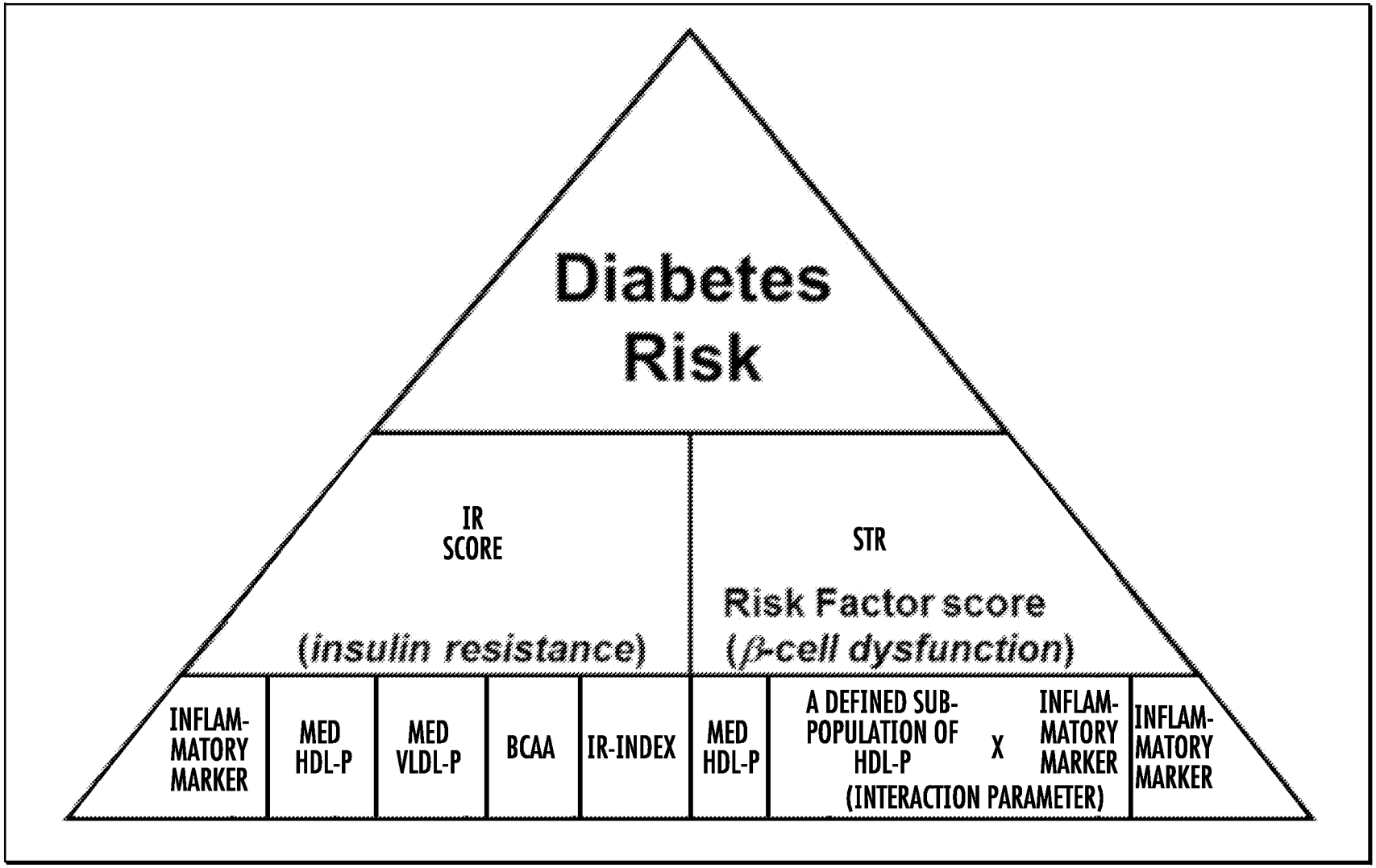

FIG. 2A

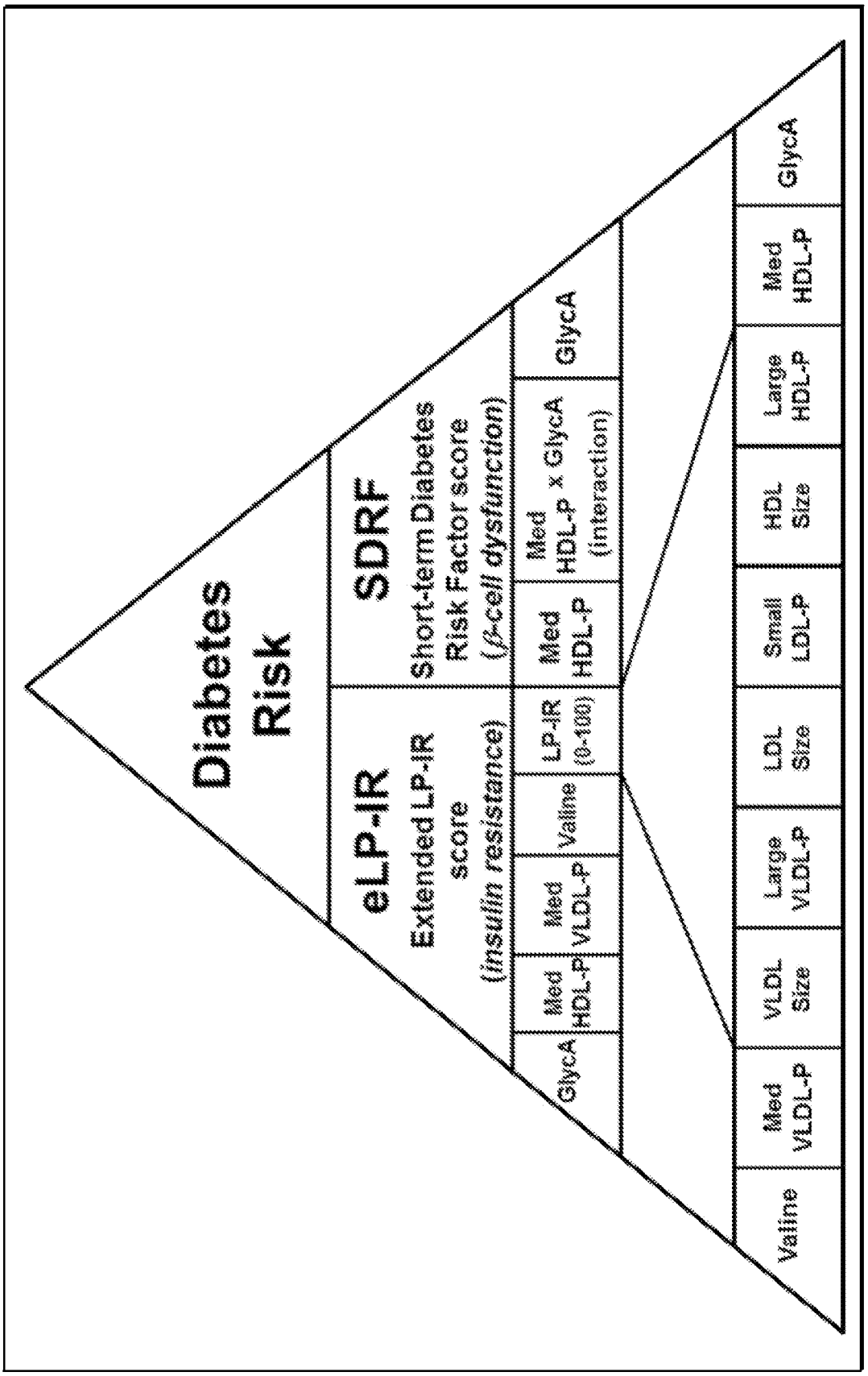
LIPOPROTEIN AND METABOLIC PARAMETERS COMPRISING eLP-IR AND SDRF SCORES
Diabetes Risk
eLP-IR
Extended LP-IR score
(insulin resistance)
SDRF
Short-term Diabetes Risk Factor score
(β-cell dysfunction)
GlycA
Med HDL-P
Med VLDL-P
Valine
LP-IR (0-100)
Med HDL-P
Med HDL-P x GlycA (interaction)
GlycA
VLDL Size
Large VLDL-P
LDL Size
Small LDL-P
HDL Size
Large HDL-P
Med HDL-P
GlycA
Valine
Med VLDL-P
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FIG. 2B

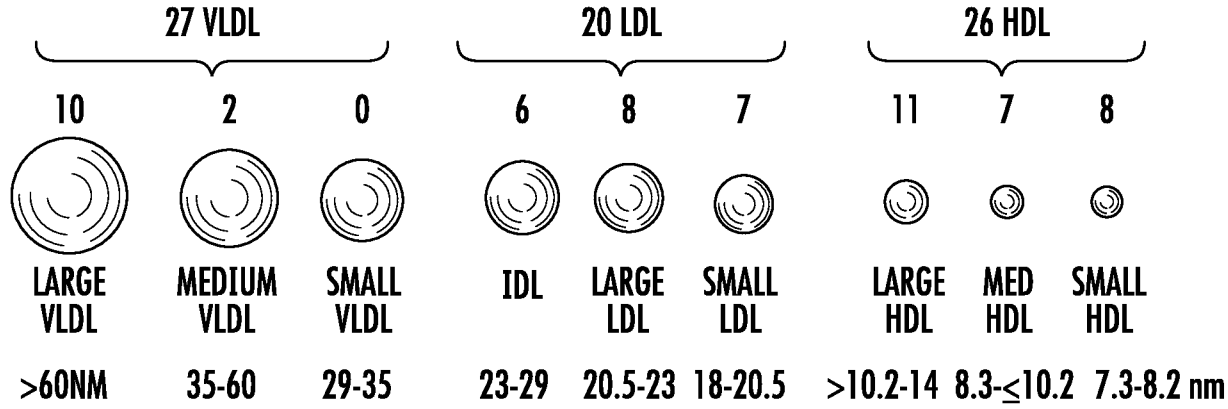

27 VLDL
20 LDL
26 HDL
10
2
0
6
8
7
11
7
8
LARGE VLDL
MEDIUM VLDL
SMALL VLDL
IDL
LARGE LDL
SMALL LDL
LARGE HDL
MED HDL
SMALL HDL
>60NM
35-60
29-35
23-29
20.5-23
18-20.5
>10.2-14
8.3-≤10.2
7.3-8.2 nm

FIG. 3A

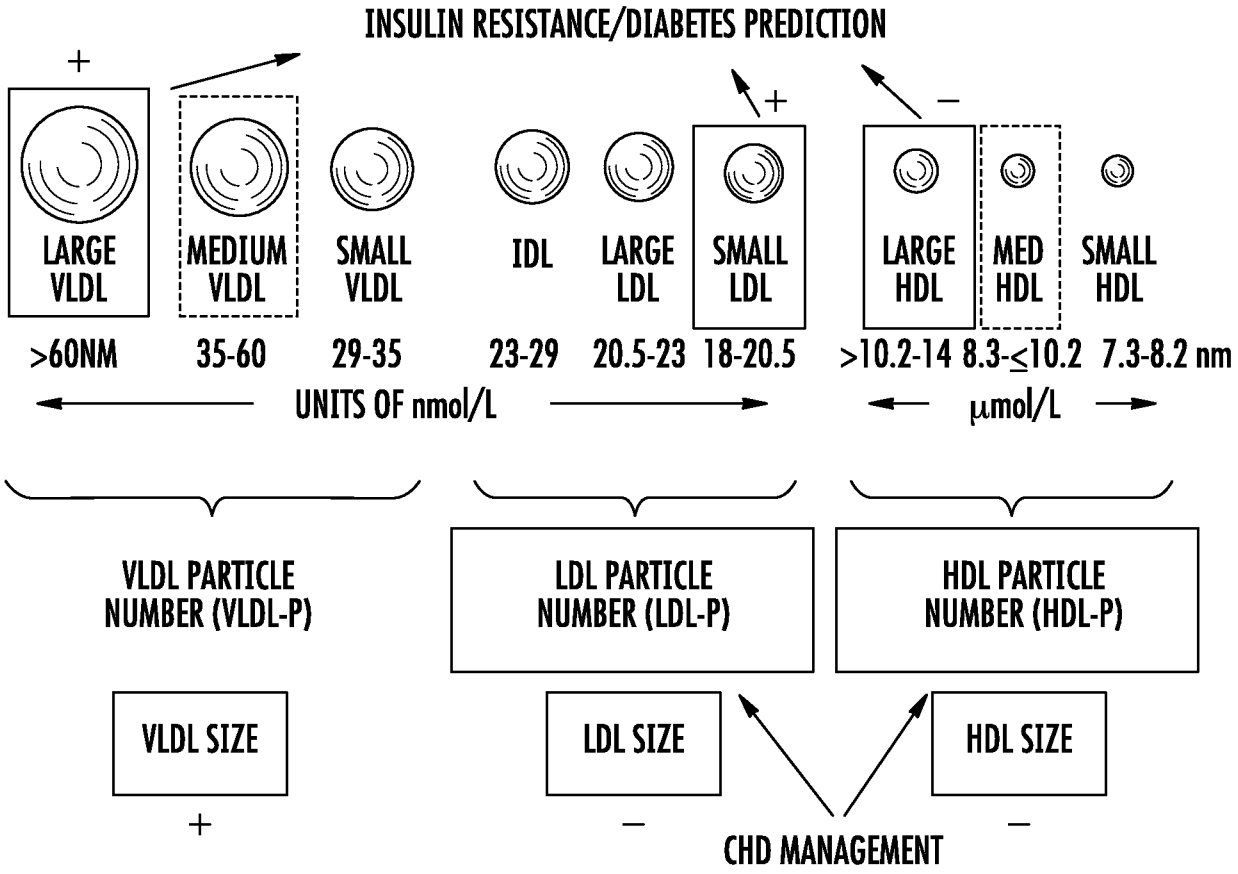

INSULIN RESISTANCE/DIABETES PREDICTION
+
+
−
LARGE VLDL
MEDIUM VLDL
SMALL VLDL
IDL
LARGE LDL
SMALL LDL
LARGE HDL
MED HDL
SMALL HDL
>60NM
35-60
29-35
23-29
20.5-23
18-20.5
>10.2-14
8.3-≤10.2
7.3-8.2 nm
UNITS OF nmol/L
μmol/L
VLDL PARTICLE NUMBER (VLDL-P)
LDL PARTICLE NUMBER (LDL-P)
HDL PARTICLE NUMBER (HDL-P)
VLDL SIZE
LDL SIZE
HDL SIZE
+
−
−
CHD MANAGEMENT

FIG. 3B

NMR HDL SUBPOPULATION GROUPINGS AND NOMENCLATURE FOR DIABETES RISK APPLICATIONS

HDL DECONVOLUTION MODEL COMPONENTS		HDL SUBPOPULATIONS			DM SUBCLASS GROUPINGS*	
COMPONENT NAME	ESTIMATED DIAMETER (nm)	COMPONENT NAME	SUBPOPULATION NAME	ESTIMATED DIAMETER (nm)	DESCRIPTIVE NAME	SUBCLASS NAMES
H1	7.4	H_{1-2}	HP1	7.4-7.5	– – –	– – –
H2	7.5					
H3	7.6	H_{3-5}	HP2	7.6-7.9	SMALL (7.6-8.2 nm)	HS_{DM}
H4	7.8					
H5	7.9					
H6	8.0	H_{6-8}	HP3	8.0-8.2		
H7	8.1					
H8	8.2					
H9	8.3	H_{9-11}	HP4	8.3-8.5	MEDIUM (8.3-10.2 nm)	HM_{DM}
H10	8.4					
H11	8.5					
H12	8.6	H_{12-14}	HP5	8.6-9.3		
H13	9.0					
H14	9.2					
H15	9.4	H_{15-17}	HP6	9.4-10.2		
H16	9.7					
H17	10.0					
H18	10.5	H_{18-20}	HP7	10.3-10.9	LARGE (>10.2 nm)	HL_{DM}
H19	10.6					
H20	10.8					
H21	11.0	H_{21-23}	HP8	11.0-12.2		
H22	11.5					
H23	12.0					
H24	12.5	H_{24-26}	HP9	12.3-13.5		
H25	13.0					
H26	13.5					

HMP: H9-H17

HMP: H9-H15

HMP: H9-H14

HMP: H9-H14 OR H9-H15 OR H9-H17

*SUBPOPULATION GROUPINGS AS GUIDED BY DIABETES RISK ASSOCIATIONS IN MESA.

FIG. 4

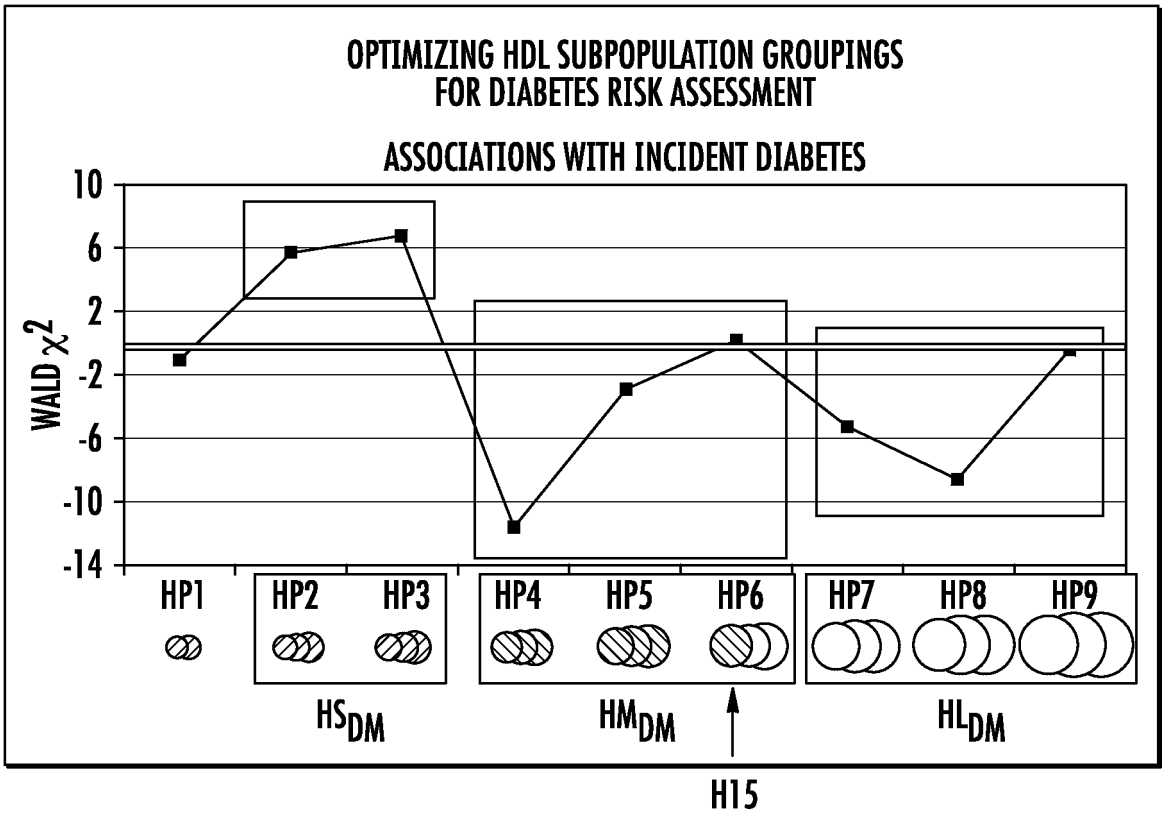

OPTIMIZING HDL SUBPOPULATION GROUPINGS
FOR DIABETES RISK ASSESSMENT
ASSOCIATIONS WITH INCIDENT DIABETES
WALD χ2
10
6
2
-2
-6
-10
-14
HP1
HP2
HP3
HP4
HP5
HP6
HP7
HP8
HP9
HSDM
HMDM
HLDM
H15

FIG. 5

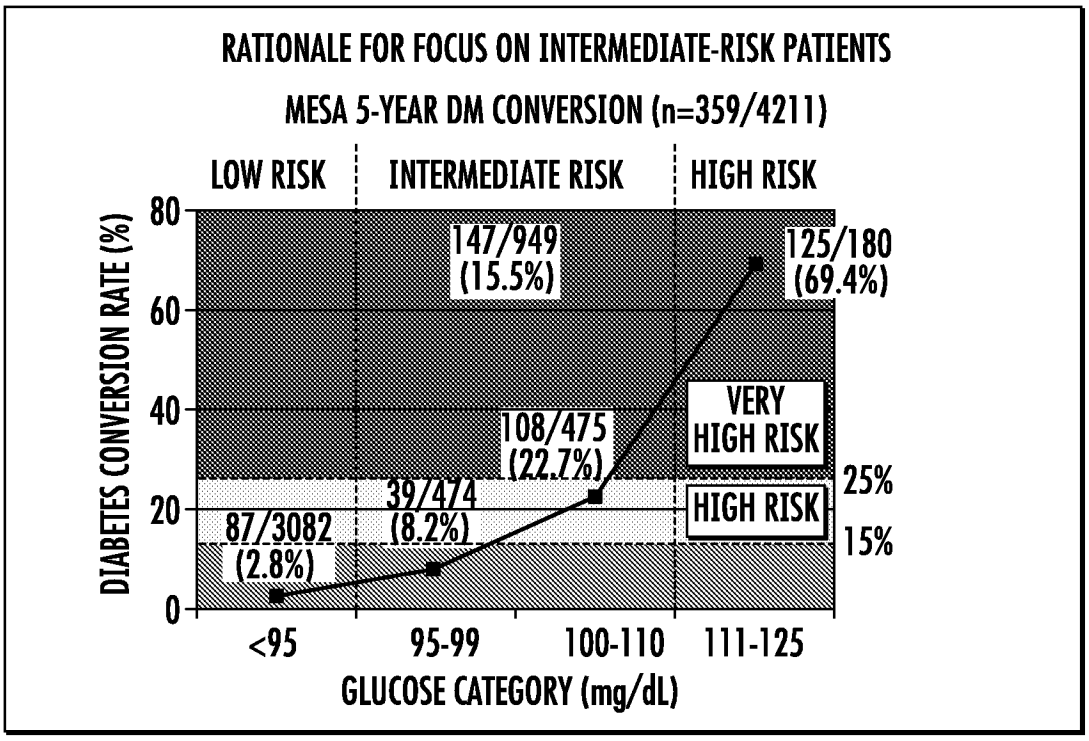


FIG. 6A

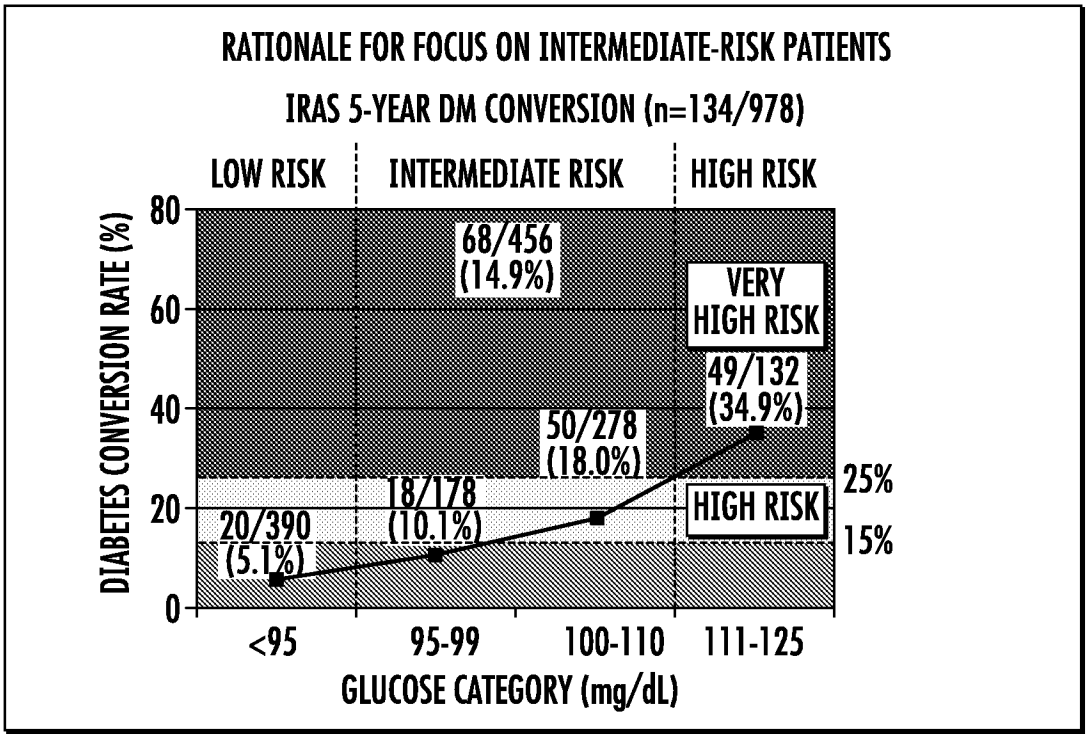


FIG. 6B

TABLE: CHARACTERISTICS OF THE MESA STUDY POPULATION (n=3450)

Characteristic	Mean (SD)	Characteristic	Mean (SD)
Age (years)	60.1 (9.6)	Fasting glucose (mg/dL)	89.0 (10.4)
Male Gender (%)	47.6	Impaired fasting glucose (%)	14.6
Race/Ethnicity (%)		Total cholesterol (mg/dL)	195 (35)
Caucasians	44.8	Triglycerides (mg/dL)	128 (74)
African Americans	22.1	HDL cholesterol (mg/dL)	51.7 (14.9)
Hispanics	20.6	Valine (μmol/L)	242 (41)
Chinese	12.5	GlycA (μmol/L)	323 (51)
BMI (kg/m^2)	28.0 (5.3)	Short-term DM converters	181
Hypertension (%)	37.4	Long-term DM converters	286

FIG. 7

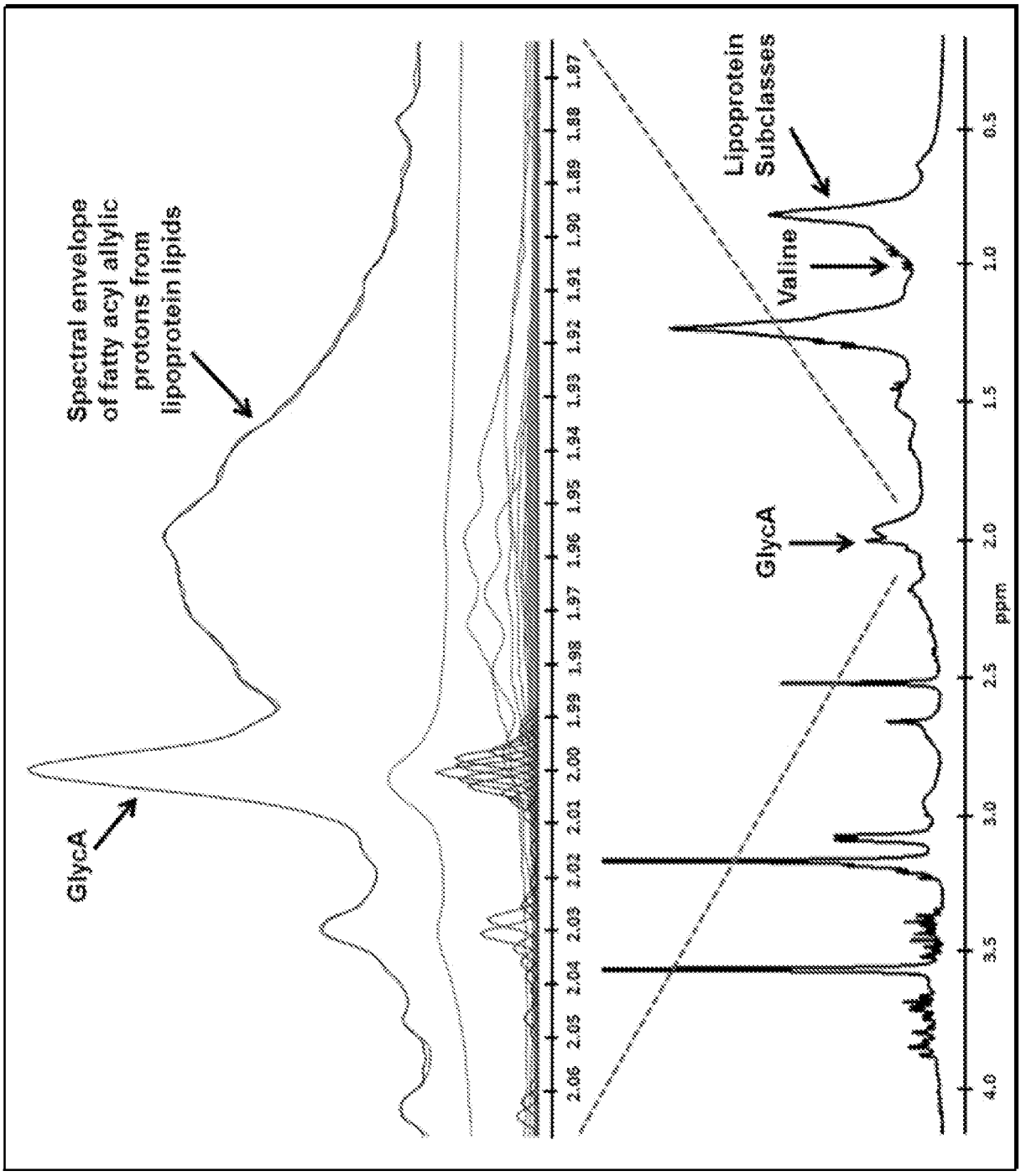
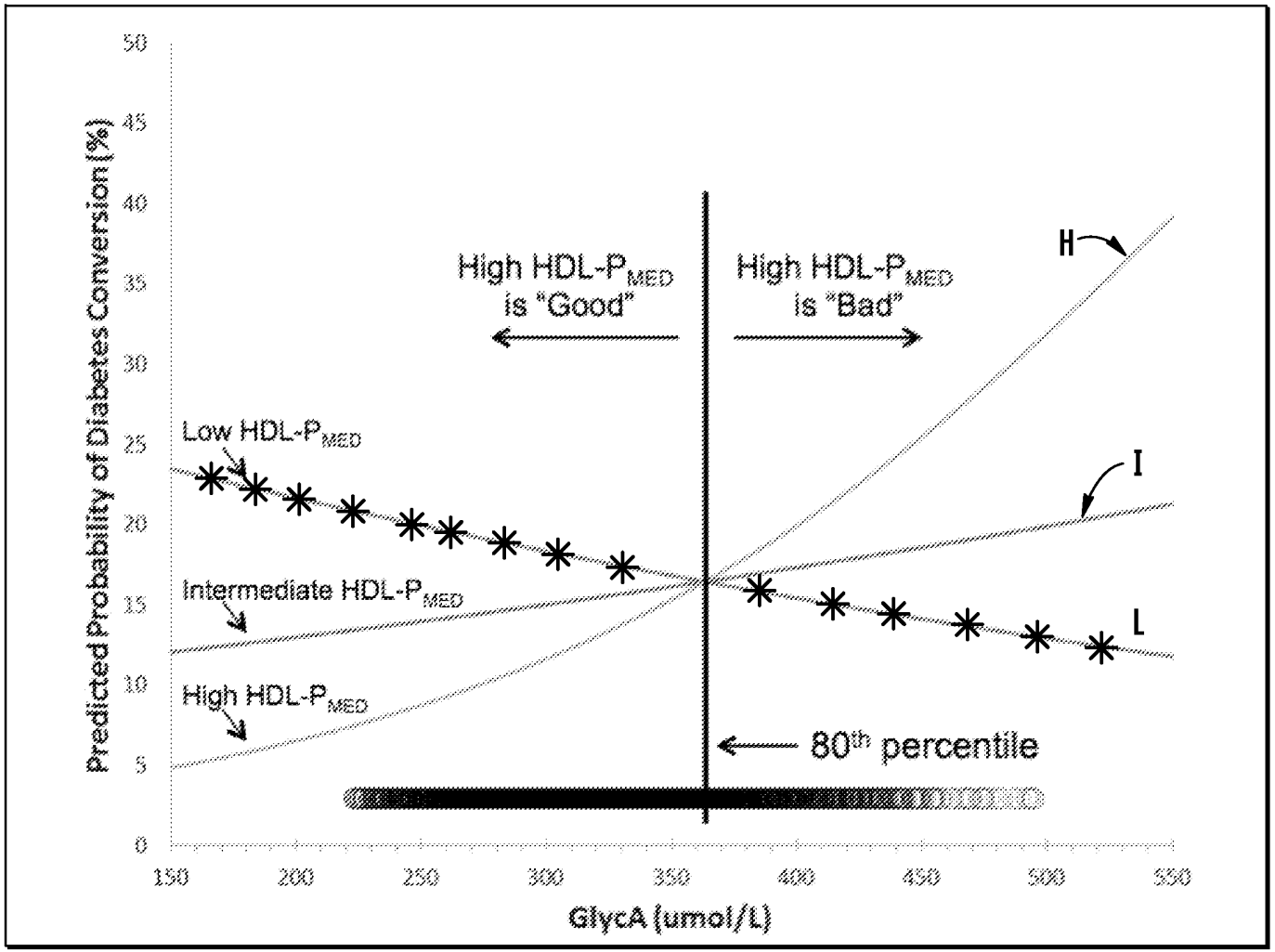

Spectral envelope of fatty acyl allylic protons from lipoprotein lipids
GlycA
GlycA
Valine
Lipoprotein Subclasses
2.06 2.05 2.04 2.03 2.02 2.01 2.00 1.99 1.98 1.97 1.96 1.95 1.94 1.93 1.92 1.91 1.90 1.89 1.88 1.87
4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

FIG. 8

PREDICTED SHORT-TERM DIABETES CONVERSION RATES BY GlycA LEVEL FOR 3 LEVELS OF HDL-P_{MED}



PREDICTED PROBABILITIES FOR SHORT-TERM CONVERSION TO DIABETES IN MESA (n=181/3450) ARE FROM LOGISTIC REGRESSION FOR A 60-YR OLD CAUCASIAN FEMALE WITH FPG=105 mg/dL, eLP-IR=0.90 (50TH PERCENTILE), AND HDL-P_{MED} VALUES OF 9.0, 12.9, or 17.7 μmol/L (25TH, 50TH, 75TH PERCENTILE).

FIG. 9

LIPOPROTEIN AND METABOLIC PARAMETERS COMPRISING eLP-IR AND SDRF SCORES

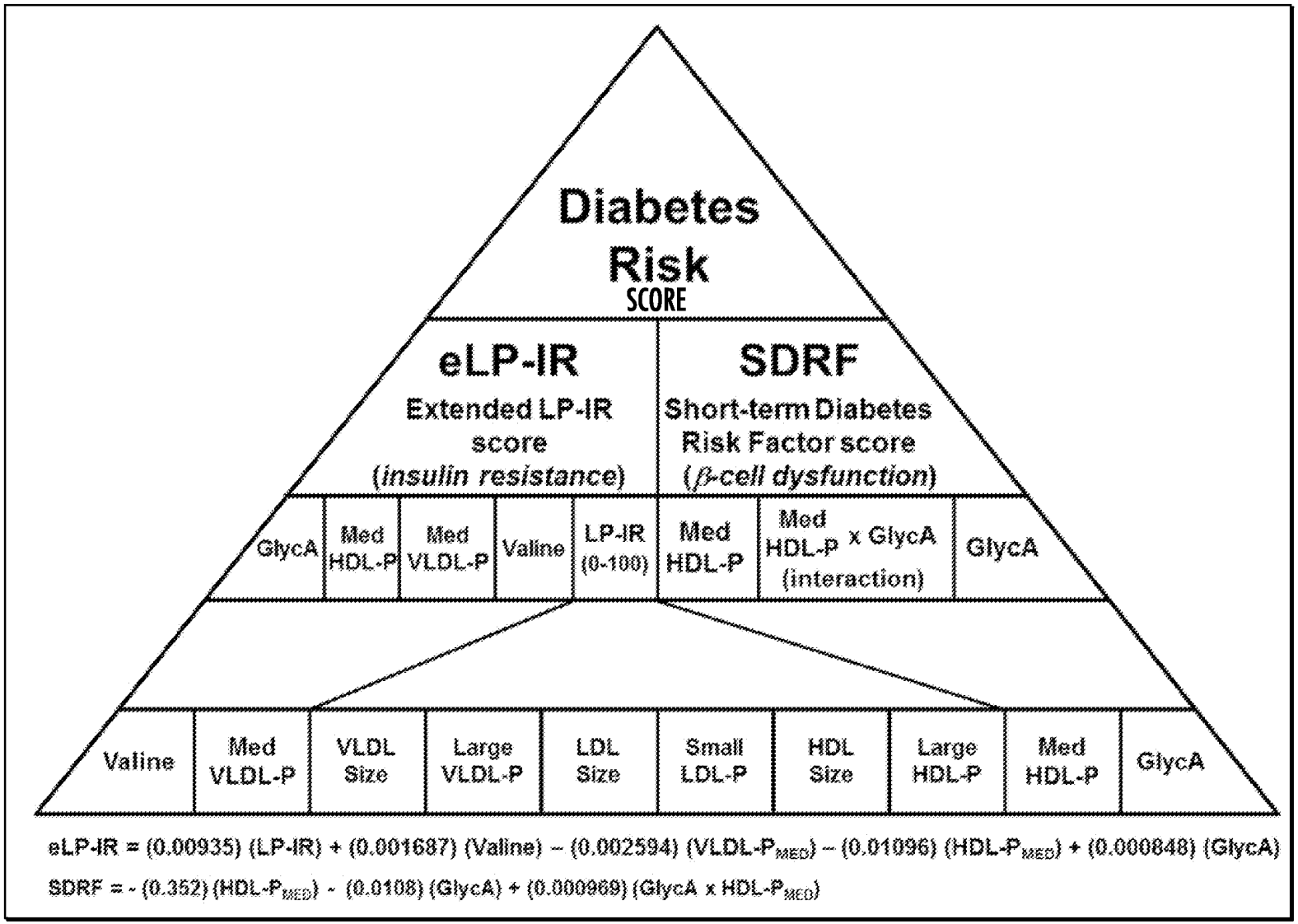


FIG. 10

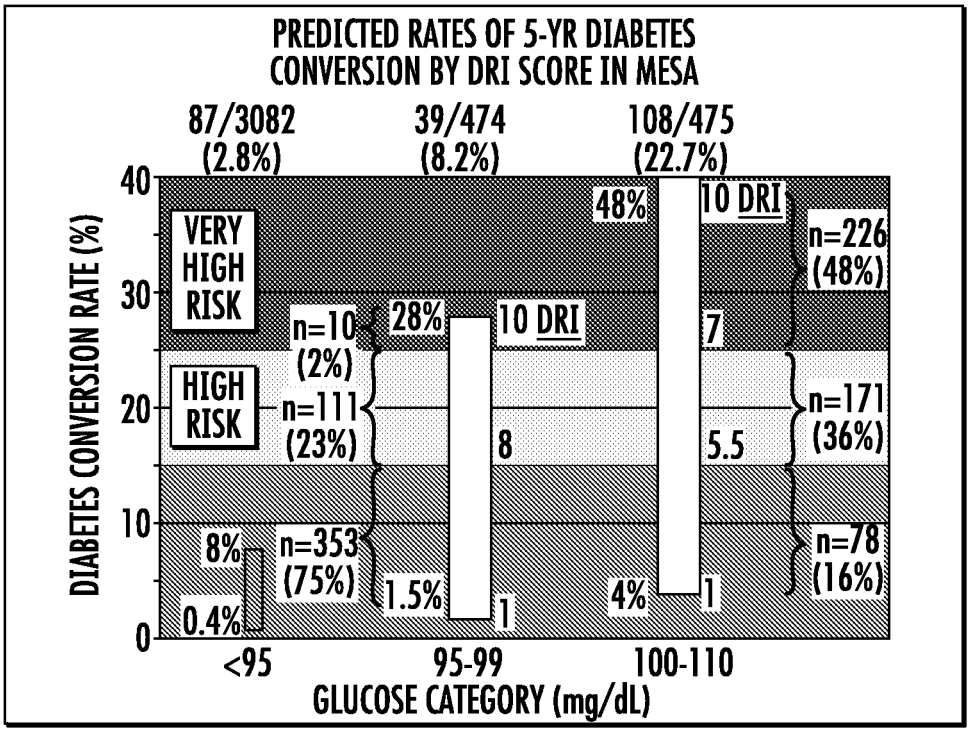


FIG. 11A

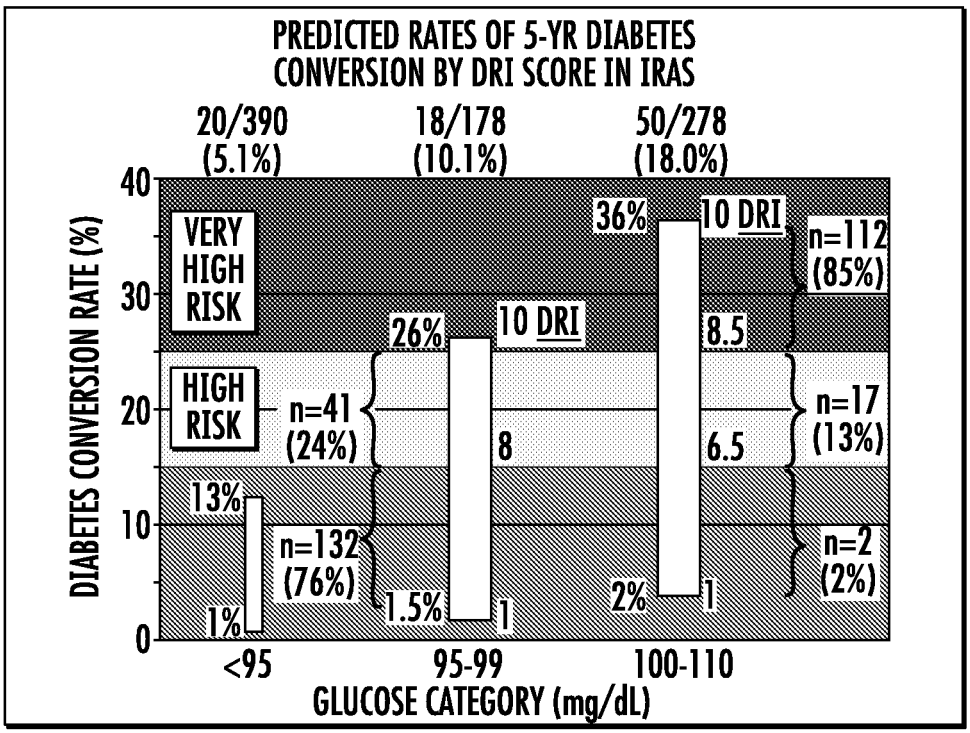


FIG. 11B

PARAMETERS CONTRIBUTING TO eLP-IR MULTIMARKER OF INSULIN RESISTANCE

Model	1-SD	Model R^2	Δ lnHOMA (SE) per 1-SD	p
Model 1 age, sex, race, glucose		0.3285	---	---
Model 2 Model 1 + 5 eLp-IR parameters		0.5050	---	---
LP-IR	23.6		0.221 (0.009)	<0.0001
Valine (µmol/L)	41.2		0.070 (0.008)	<0.0001
Med VLDL-P (nmol/L)	21.4		-0.056 (0.008)	<0.0001
Med HDL-P (µmol/L)	6.5		-0.071 (0.007)	<0.0001
GlycA (µmol/L)	51.1		0.043 (0.007)	<0.0001
Model 3 Model 1 + eLP-IR		0.5050		
eLP-IR	0.26		0.261 (0.007)	<0.0001

FROM MULTIPLE LINEAR REGRESSION IN MESA (n=3446) WITH INSULIN RESISTANCE (lnHOMA-IR) AS DEPENDENT VARIABLE. STRENGTHS OF ASSOCIATION OF EACH VARIABLE WITH INSULIN RESISTANCE ARE GIVEN AS THE DIFFERENCE IN ln(HOMA-IR) PER 1-SD INCREMENT.
eLP-IR WAS DERIVED AS FOLLOWS USING BETA-COEFFICIENTS FROM MODEL 2:
eLP-IR = (0.00935) (LP-IR) + (0.001687) (VALINE) – (0.002594) (VLDL-P_{MED}) – (0.01096) (HDL-P_{MED}) + (0.000848) (GlycA)

FIG. 12

PARAMETERS CONTRIBUTING TO SDRF (SHORT-TERM DIABETES RISK FACTOR) SCORE

	Short-term Diabetes Conversion Diabetes at Visits 2 or 3 (mean 1.8 yrs; n=181/3450)				Long-term Diabetes Conversion Diabetes at Visits 4 or 5 (mean 7.8 yrs;n=286/3269)			
Logistic Regression Model	**LRχ^2**	**AUC**	**OR**	**p**	**LRχ^2**	**AUC**	**OR**	**p**
Model 1 age, sex, race, glucose	523.8	0.896	---	---	273.9	0.763	---	---
Model 2 Model 1 + HOMA-IR	538.1	0.904	---	---	314.1	0.793	---	---
HOMA-IR			1.38	0.003			1.63	<0.0001
Model 3 Model 1 + eLP-IR	555.4	0.911	---	---	325.1	0.795	---	---
eLP-IR			1.85	<0.0001			1.73	<0.0001
Model 4 Model 3 + SDRF components	567.9	0.914	---	---	325.9	0.796	---	---
eLP-IR			1.76	<0.0001			1.68	<0.0001
HDL-P_{MED}			(-)	0.0004			(+)	0.61
GlycA			(-)	0.009			(+)	0.45
HDL-P_{MED} x GlycA			(+)	0.0006			(-)	0.55
Model 5 Model 1 + eLp-IR + SDRF	567.9	0.914	---	---	325.2	0.795	---	---
eLP-IR			1.77	<0.0001			1.74	<0.0001
SDRF			1.50	0.0004			0.98	0.76

FROM LOGISTIC REGRESSION IN MESA WITH SHORT-TERM (n=181/3450) OR LONG-TERM (n=286/3269) DIABETES CONVERSION AS DEPENDENT VARIABLE. STRENGTHS OF ASSOCIATION ARE GIVEN BY THE ODDS RATIO (OR) PER 1-SD INCREMENT. SDRF WAS DERIVED AS FOLLOWS USING BETA-COEFFICIENTS FROM MODEL 4 FOR SHORT-TERM DIABETES CONVERSION:

SDRF = - (0.352) (HDL-P_{MED}) - (0.0108) (GlycA) + (0.000969) (GlycA x HDL-P_{MED})

 FIG. 13

eLP-IR AND SDRF PREDICT 5-YEAR DIABETES CONVERSION IN IRAS (INSULIN RESISTANCE ATHEROSCLEROSIS STUDY)

	Conversion to Diabetes During 5.2-Year Follow-up in IRAS (n=134/976)			
Model	**LRχ^2**	**AUC**	**OR**	**p**
Model 1 age, sex, race, glucose	92.6	0.749	2.49	<0.0001
Model 2 Model 1 + Si	100.0	0.765	---	---
Si			0.62	<0.0001
Model 3 Model 1 + LP-IR	119.9	0.774	---	---
LP-IR			1.82	<0.0001
Model 4 Model 1 + eLP-IR	124.2	0.780	---	---
eLP-IR			1.91	<0.0001
Model 5 Model 4 + SDRF	129.3	0.787	---	---
eLP-IR			1.90	<0.0001
SDRF			1.27	0.02

FROM LOGISTIC REGRESSION IN IRAS WITH 5-YEAR DIABETES CONVERSION AS DEPENDENT VARIABLE. RELATIVE PREDICTIVE VALUES OF THE 5 REGRESSION MODELS ARE GIVEN BY THE LIKELIHOOD RATIO (LR) χ2 STATISTIC AND AREA UNDER THE ROC CURVE (AUC). STRENGTHS OF ASSOCIATION OF THE INDICATED VARIABLES ARE GIVEN BY THE ODDS RATIO (OR) PER 1-SD INCREMENT. S_i IS INSULIN SENSITIVITY MEASURED BY FREQUENTLY SAMPLED INTRAVENOUS GLUCOSE TOLERANCE TESTING.

FIG. 14

MEANING OF HDL-P_{MED} x GlycA INTERACTION IN IRAS

RATES OF 5-Yr DIABETES CONVERSION IN IRAS BY TERTILE OF GlycA AND HDL-P_{MED} TERTILE

		GlycA Tertile		
		Low	Intermed	High
HDL-P_{MED} Tertile	Low	10.1% n=109	13.3% n=98	12.9% n=70
	Intermed	7.1% n=99	7.7% n=91	11.8% n=93
	High	2.3% n=86	9.3% n=97	17.8% n=107

DIABETES CONVERSION RATES BY TERTILE OF HDL-P_{MED} AND GlycA IN IRAS PARTICIPANTS WITH FASTING GLUCOSE ≤110 mg/dL (n=88/850).

FIG. 15

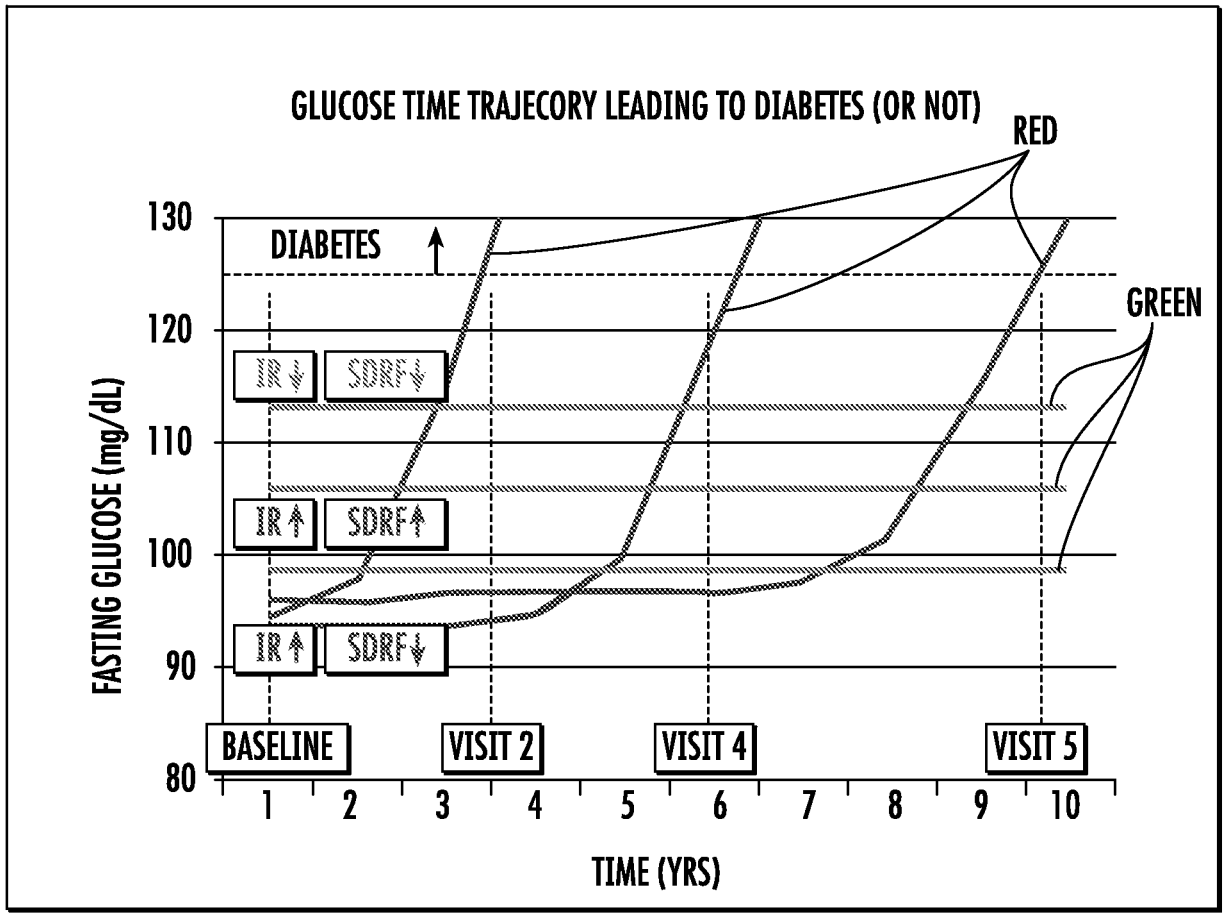


FIG. 16

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SUITE 140
RALEIGH, NC
PHONE: (919)256-1111 FAX: (919)256-1221

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			TESTREQ1234	FASTING

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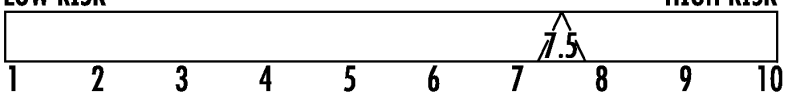
DIABETES RISK INDEX 7.5



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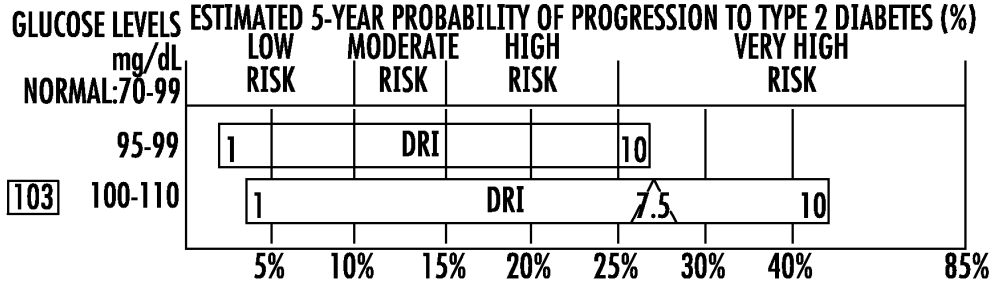
DIABETES RISK INTERPRETATION WITH GLUCOSE RESULTS



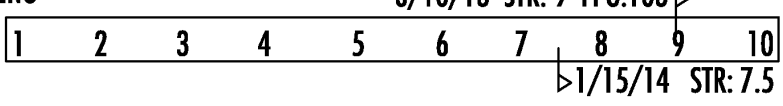
5 YEAR RISK CATEGORY: VERY HIGH — YOU HAVE AN ESTIMATED 27% PROBABILITY OF PROGRESSING TO TYPE 2 DIABETES WITHIN 5 YEARS.

CARDIOVASCULAR IMPLICATIONS OF DIABETES RISK

PATIENTS WITH AN ELEVATED DRI ARE LIKELY TO HAVE HIGHER LDL PARTICLE NUMBER (LDL-P), AND INCREASED LDL-RELATED CARDIOVASCULAR RISK, DESPITE NORMAL OR DECREASED LDL CHOLESTEROL (LDL-C) VALUES. CONSIDER MEASURING LDL-P BY THE NMR LIPOPROFILE® TEST TO AID IN THE MANAGEMENT OF LDL-RELATED CARDIOVASCULAR RISK.

HISTORICAL REPORTING

STR (β CELL DYSFUNCTION)



ADDITIONAL INFORMATION

	μmol//L		μmol//L
GlycA	400	VALINE	260
REF. RANGE:	243-498	REF. RANGE:	150-333

GlycA, A MARKER OF SYSTEMIC INFLAMMATION, AND VALINE, A BRANCHED-CHAIN AMINO-ACID, HAVE STRONG CLINICAL ASSOCIATIONS WITH PROGRESSION TO TYPE 2 DIABETES

THESE LABORATORY ASSAYS, VALIDATED BY LIPOSCIENCE, HAVE NOT BEEN CLEARED BY THE US FOOD AND DRUG ADMINISTRATION. THE CLINICAL UTILITY OF THESE LABORATORY VALUES HAVE NOT BEEN FULLY ESTABLISHED.

FIG. 17

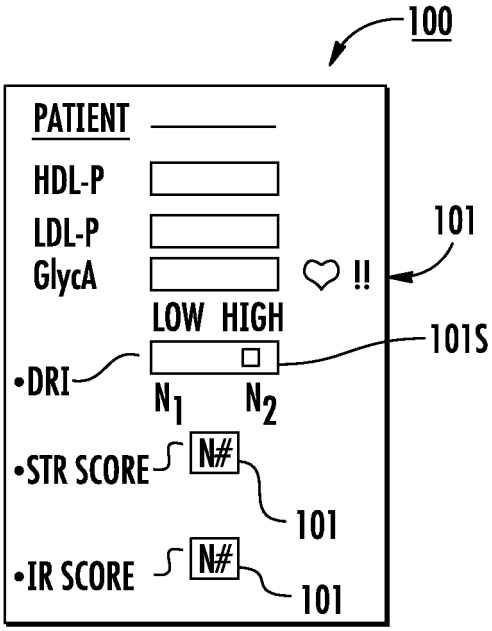

100
PATIENT
HDL-P
LDL-P
GlycA
!!
101
LOW HIGH
101S
•DRI
N1 N2
•STR SCORE
N#
101
•IR SCORE
N#
101

FIG. 18

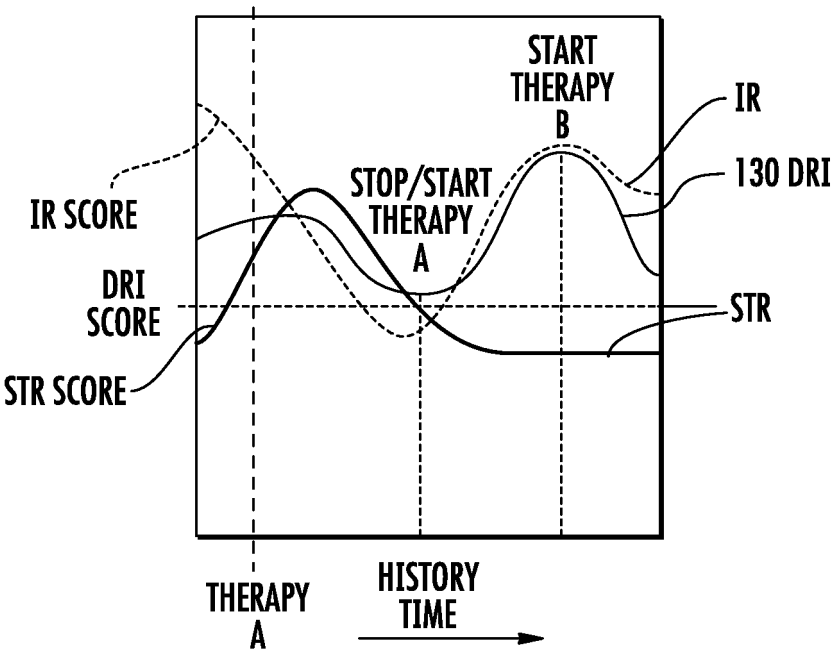

START THERAPY B
IR
130 DRI
STOP/START THERAPY A
IR SCORE
DRI SCORE
STR
STR SCORE
THERAPY A
HISTORY TIME

FIG. 19

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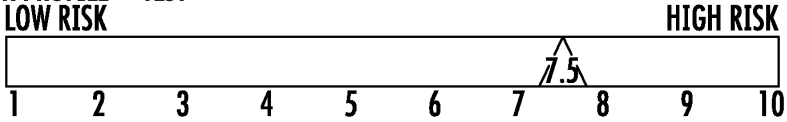
			TESTREQ1234	FASTING

NMR DIABETES RISK PROFILE® TEST

DIABETES RISK INDEX 7.5



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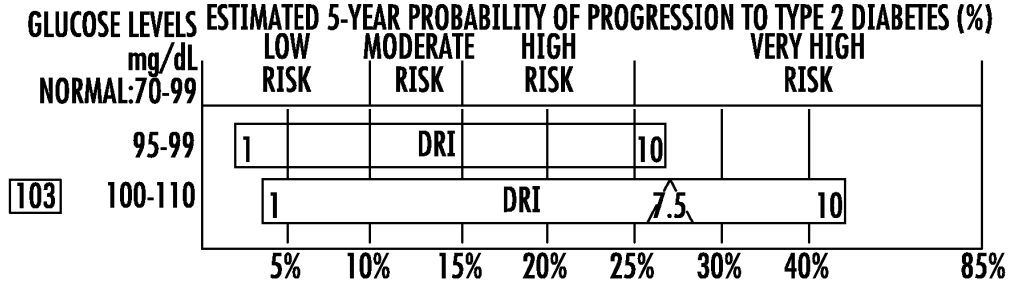
DIABETES RISK INTERPRETATION WITH GLUCOSE RESULTS



5 YEAR RISK CATEGORY: VERY HIGH — YOU HAVE AN ESTIMATED 27% PROBABILITY OF PROGRESSING TO TYPE 2 DIABETES WITHIN 5 YEARS.

CARDIOVASCULAR IMPLICATIONS OF DIABETES RISK

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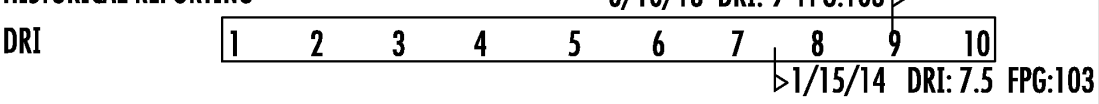
HISTORICAL REPORTING



ADDITIONAL INFORMATION

	μmol//L		μmol//L
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REF. RANGE:	243-498	REF. RANGE:	150-333

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FIG. 20A

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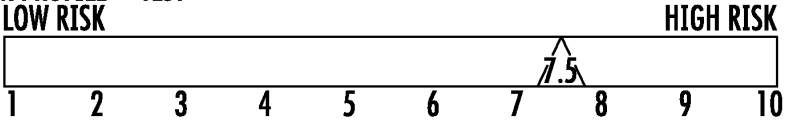
			TESTREQ1234	FASTING

NMR DIABETES RISK PROFILE® TEST

DIABETES RISK INDEX: 7.5



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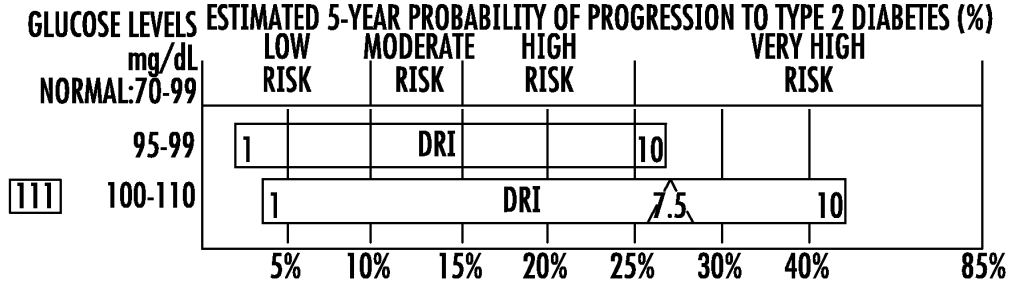
DIABETES RISK INTERPRETATION WITH GLUCOSE RESULTS



5 YEAR RISK CATEGORY: HIGH

YOUR FASTING GLUCOSE IS ABOVE 110 mg/dL, AND YOUR PROBABILITY OF PROGRESSING TO TYPE 2 DIABETES WITHIN 5 YEARS IS > 15%.

CARDIOVASCULAR IMPLICATIONS OF DIABETES RISK

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HISTORICAL REPORTING

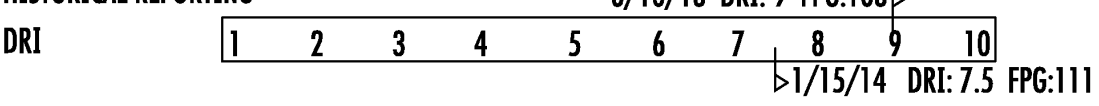

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FIG. 20B

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PATIENT ID	BIRTH DATE	ACCESSION NUMBER
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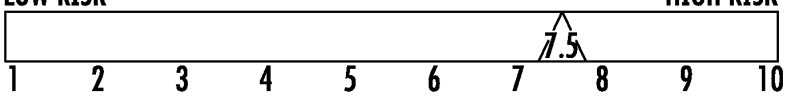
DATE COLLECTED	DATE RECEIVED	REPORT DATE AND TIME	REQUISITION NUMBER	FASTING STATUS
			TESTREQ1234	FASTING

NMR DIABETES RISK PROFILE® TEST

DIABETES RISK INDEX 7.5



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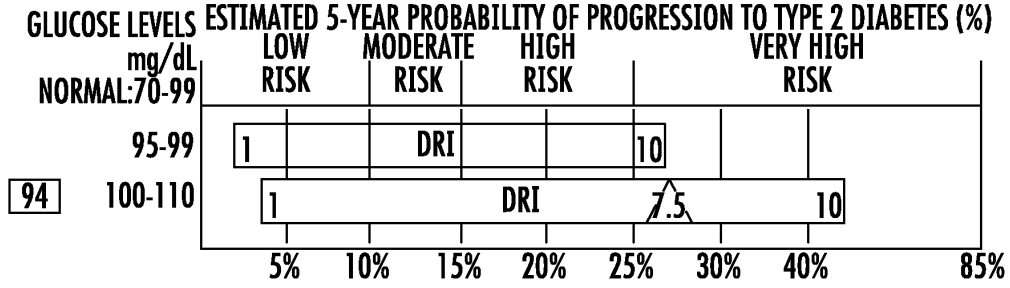
DIABETES RISK INTERPRETATION WITH GLUCOSE RESULTS



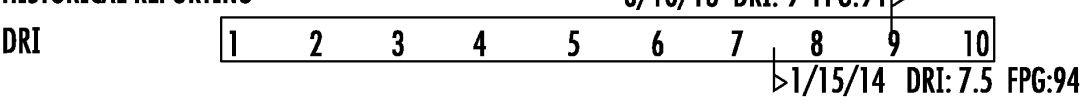
5 YEAR RISK CATEGORY: LOW

YOUR FASTING GLUCOSE IS BELOW 95 mg/dL, AND YOUR PROBABILITY OF PROGRESSING TO TYPE 2 DIABETES WITHIN 5 YEARS IS < 10%.

CARDIOVASCULAR IMPLICATIONS OF DIABETES RISK

PATIENTS WITH AN ELEVATED DRI ARE LIKELY TO HAVE HIGHER LDL PARTICLE NUMBER (LDL-P), AND INCREASED LDL-RELATED CARDIOVASCULAR RISK, DESPITE NORMAL OR DECREASED LDL CHOLESTEROL (LDL-C) VALUES. CONSIDER MEASURING LDL-P BY THE NMR LIPOPROFILE® TEST TO AID IN THE MANAGEMENT OF LDL-RELATED CARDIOVASCULAR RISK.

HISTORICAL REPORTING



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	μmol//L		μmol//L
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FIG. 20C

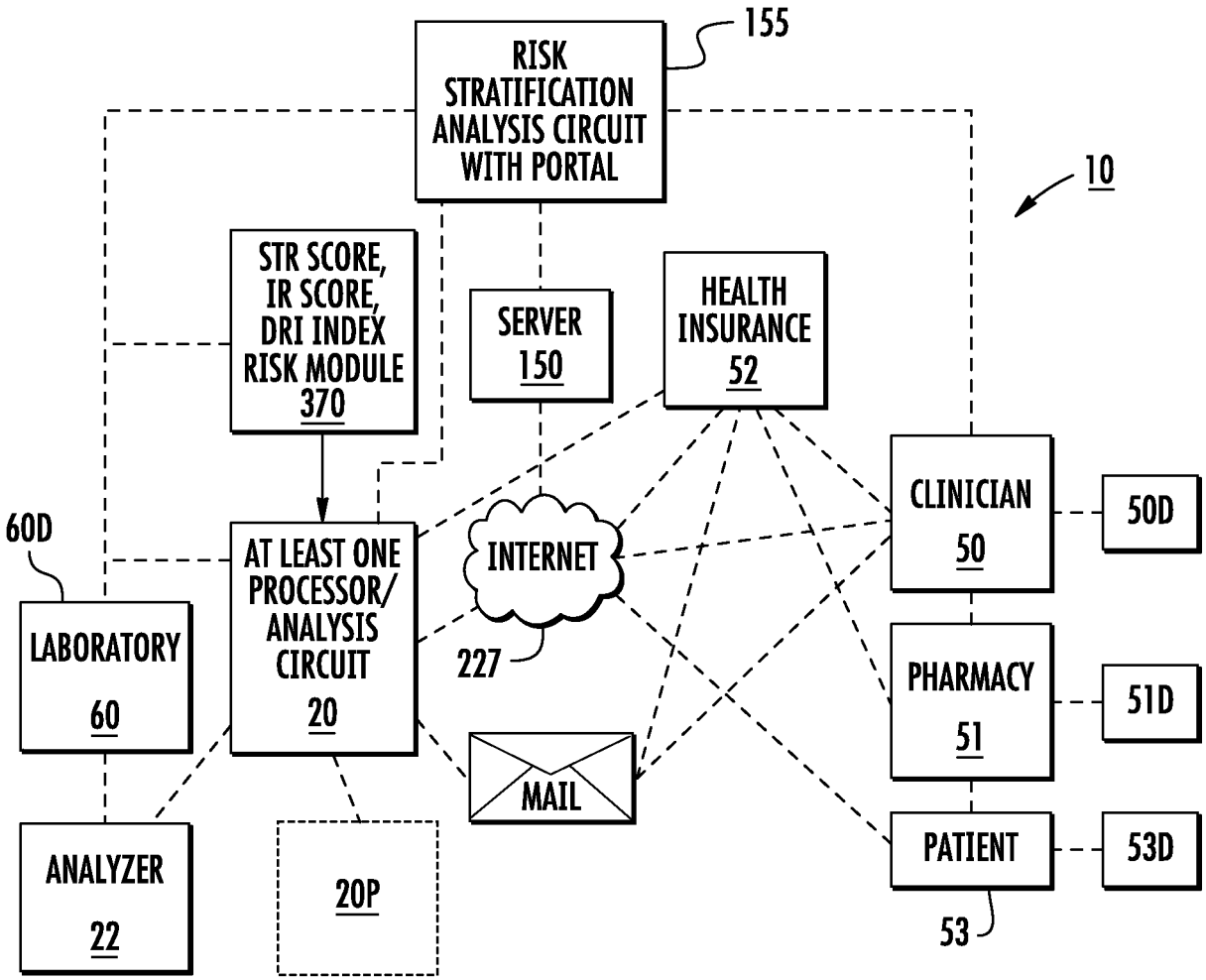
RISK STRATIFICATION ANALYSIS CIRCUIT WITH PORTAL
155
10
STR SCORE, IR SCORE, DRI INDEX RISK MODULE 370
SERVER 150
HEALTH INSURANCE 52
CLINICIAN 50
50D
60D
AT LEAST ONE PROCESSOR/ ANALYSIS CIRCUIT 20
INTERNET
227
LABORATORY 60
PHARMACY 51
51D
MAIL
ANALYZER 22
20P
PATIENT
53D
53

FIG. 21

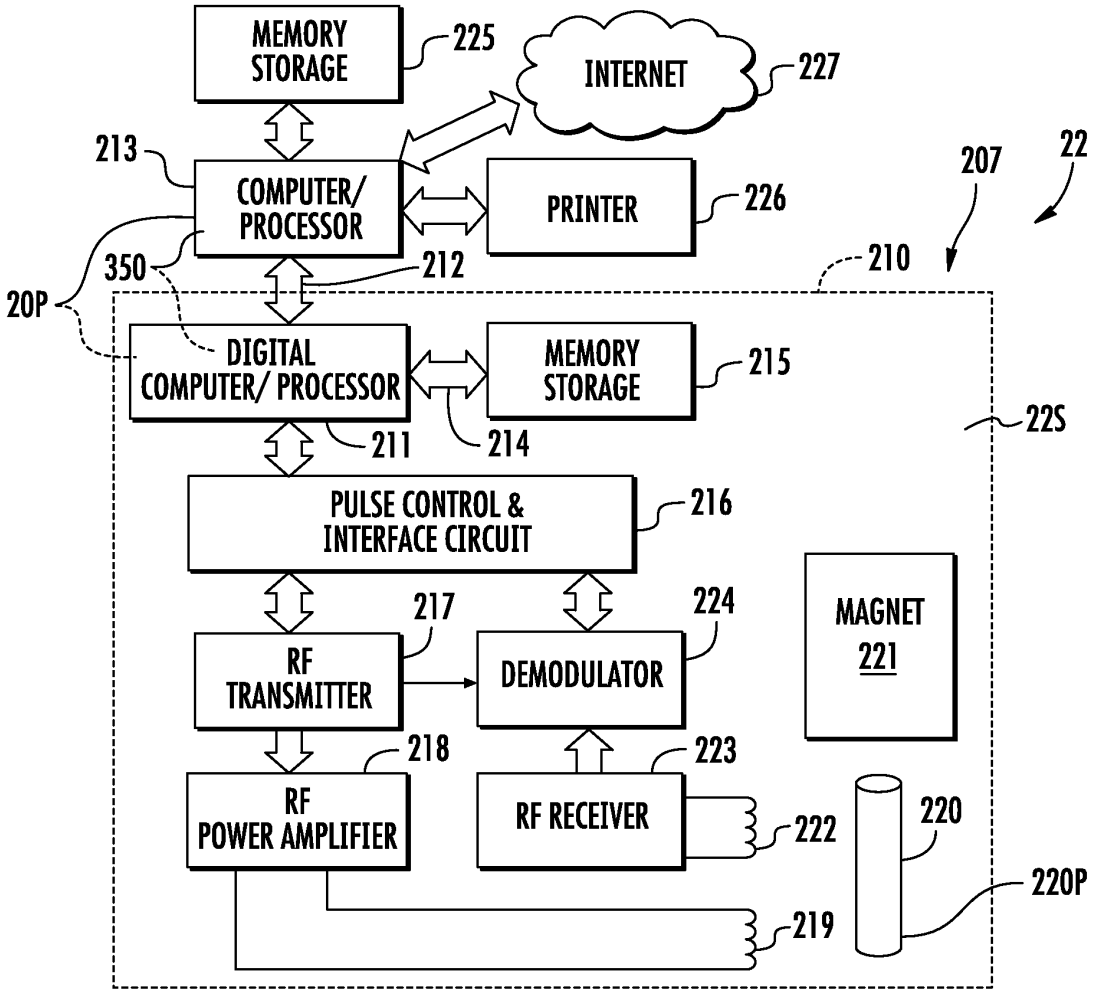
MEMORY STORAGE
225
INTERNET
227
213
COMPUTER/ PROCESSOR
PRINTER
226
207
22
350
212
210
20P
DIGITAL COMPUTER/ PROCESSOR
MEMORY STORAGE
215
22S
211
214
PULSE CONTROL & INTERFACE CIRCUIT
216
MAGNET
221
217
224
RF TRANSMITTER
DEMODULATOR
218
223
RF POWER AMPLIFIER
RF RECEIVER
222
220
220P
219

FIG. 22

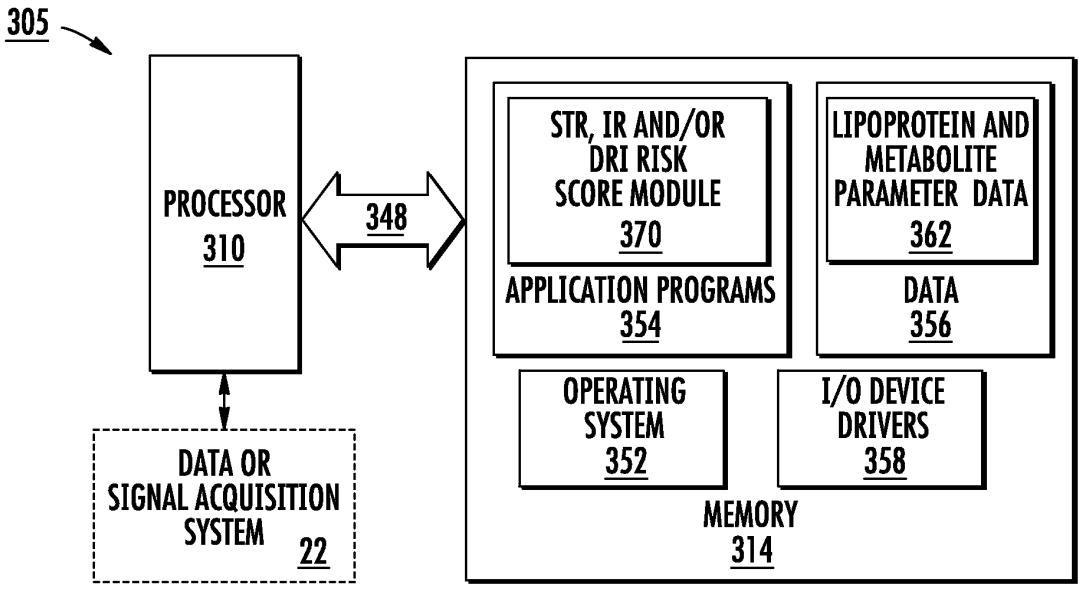
305
PROCESSOR
310
348
STR, IR AND/OR DRI RISK SCORE MODULE
370
LIPOPROTEIN AND METABOLITE PARAMETER DATA
362
APPLICATION PROGRAMS
354
DATA
356
OPERATING SYSTEM
352
I/O DEVICE DRIVERS
358
MEMORY
314
DATA OR SIGNAL ACQUISITION SYSTEM
22

FIG. 23

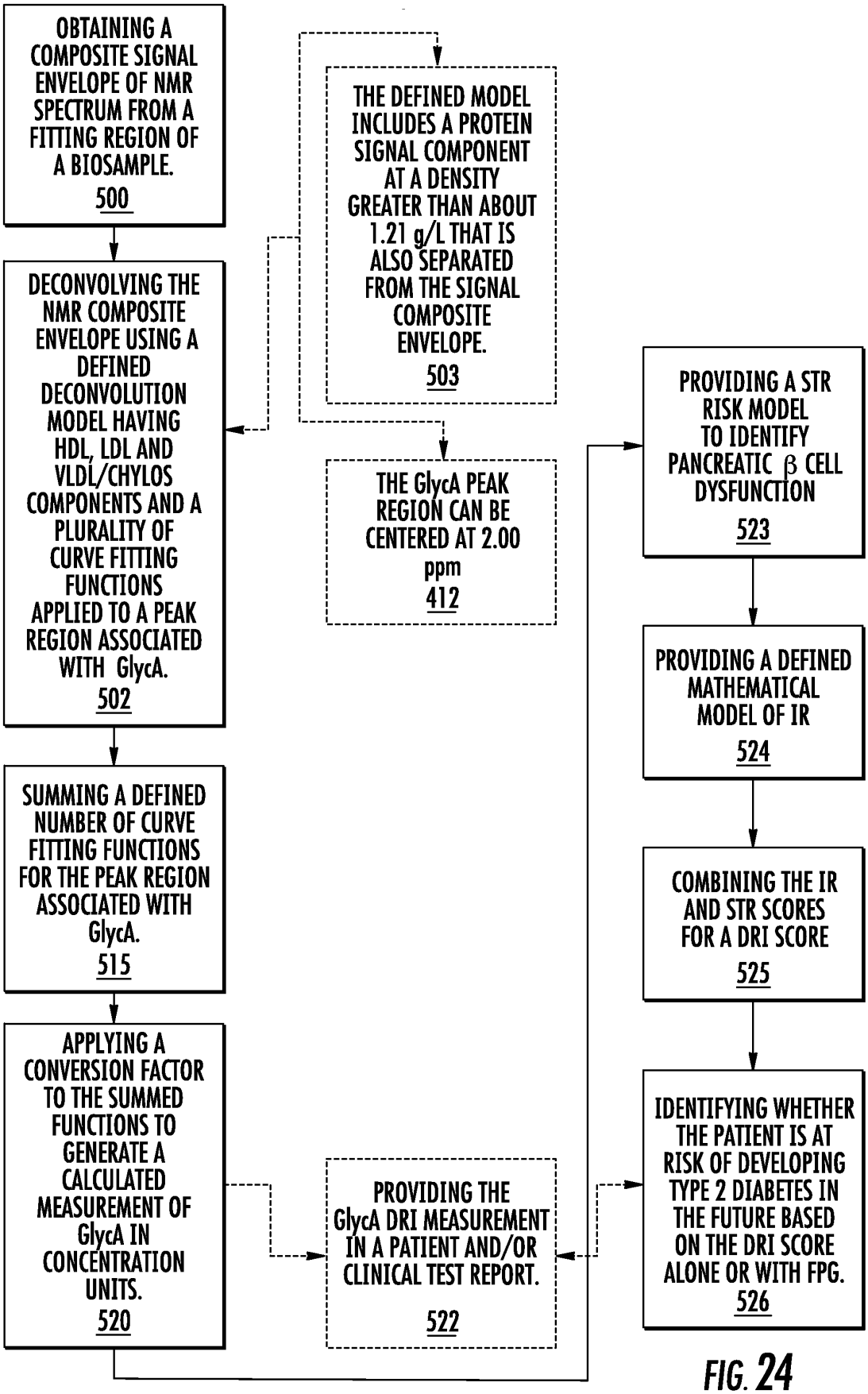
OBTAINING A COMPOSITE SIGNAL ENVELOPE OF NMR SPECTRUM FROM A FITTING REGION OF A BIOSAMPLE.
500
DECONVOLVING THE NMR COMPOSITE ENVELOPE USING A DEFINED DECONVOLUTION MODEL HAVING HDL, LDL AND VLDL/CHYLOS COMPONENTS AND A PLURALITY OF CURVE FITTING FUNCTIONS APPLIED TO A PEAK REGION ASSOCIATED WITH GlycA.
502
SUMMING A DEFINED NUMBER OF CURVE FITTING FUNCTIONS FOR THE PEAK REGION ASSOCIATED WITH GlycA.
515
APPLYING A CONVERSION FACTOR TO THE SUMMED FUNCTIONS TO GENERATE A CALCULATED MEASUREMENT OF GlycA IN CONCENTRATION UNITS.
520
THE DEFINED MODEL INCLUDES A PROTEIN SIGNAL COMPONENT AT A DENSITY GREATER THAN ABOUT 1.21 g/L THAT IS ALSO SEPARATED FROM THE SIGNAL COMPOSITE ENVELOPE.
503
THE GlycA PEAK REGION CAN BE CENTERED AT 2.00 ppm
412
PROVIDING THE GlycA DRI MEASUREMENT IN A PATIENT AND/OR CLINICAL TEST REPORT.
522
PROVIDING A STR RISK MODEL TO IDENTIFY PANCREATIC β CELL DYSFUNCTION
523
PROVIDING A DEFINED MATHEMATICAL MODEL OF IR
524
COMBINING THE IR AND STR SCORES FOR A DRI SCORE
525
IDENTIFYING WHETHER THE PATIENT IS AT RISK OF DEVELOPING TYPE 2 DIABETES IN THE FUTURE BASED ON THE DRI SCORE ALONE OR WITH FPG.
526

FIG. 24

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 4933844 A **[0007]**
- US 6617167 B **[0007]**
- US 6518069 B, Otvos **[0007]**
- US 8386187 B **[0009] [0100]**
- US 83078413 **[0072]**
- US 2013044679 W **[0072]**
- WO 2013185014 A **[0072]**
- US 2013064142 W **[0105]**
- WO 2014059025 A **[0105]**
- US 20090155826 A, Hu **[0106]**
- US 8013602 B **[0171]**

Non-patent literature cited in the description

- **MASON et al.** *Diabetes,* 2007, vol. 56, 2054-6 **[0010]**
- AACE Prediabetes Consensus Statement. *Endocr Pract,* 2008, vol. 14 (7), 941 **[0010]**
- Standards of medical care in diabetes-2012. Diabetes Care. American Diabetes Association, 2012, vol. 35, S12 **[0075]**
- **SHALAUROVA I et al.** Lipoprotein Insulin Resistance Index: A Lipoprotein Particle-Derived Measure of Insulin Resistance. *Metabolic Syndrome and Related Disorders,* October 2014, vol. 12 (8), 422-429 **[0100]**
- **DREW et al.** The Emerging Roles of HDL in Glucose Metabolism. *Nat. Rev., Endocrinol.,* 24 January 2012, vol. 8, 237-245 **[0102]**
- **TABAK AG et al.** *Lancet,* 2009, vol. 373, 2215-21 **[0103]**
- **KAESS et al.** The lipoprotein subfraction profile: heritability and identification of quantitative trait loci. *J Lipid Res.,* 2008, vol. 49, 715-723 **[0121]**
- **SUNA et al.** 1H NMR metabolomics of plasma lipoprotein subclasses: elucidation of metabolic clustering by self-organizing maps. *NMR Biomed.,* 2007, vol. 20, 658-672 **[0121]**
- **MARTIN et al.** High-density lipoprotein subfractions: Current views and clinical practice applications. *Trends Endocrinol Metab,* 2014, vol. 25, 329-336 **[0121]**
- **JEYARAJAH et al.** Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin Lab Med.,* 2006, vol. 26, 847-870 **[0123]**

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[标]发明人	OTVOS JAMES D SHALAUROVA IRINA Y		
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摘要(译)

方法，系统和电路使用确定的短期风险（STR）和长期进展风险的数学模型评估受试者患2型糖尿病的风险。评估可以对具有相同葡萄糖测量值的患者的风险进行分层，特别是具有中等或低（正常）空腹血糖（FPG）值的患者。 STR或IR（胰岛素抗性）模型可以包括炎性生物标志物，例如NMR衍生的GlycA测量值和受试者的至少一个生物样品的多种选定的脂蛋白组分。本发明的实施方案还提供产生STR评分作为β细胞功能障碍或损伤的标志物的方法，系统和电路。