METHODS OF DETERMINING IMPAIRED GLUCOSE TOLERANCE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of United States Provisional Application No. 62/959,660, filed January 10, 2020, which is incorporated by reference herein in its entirety for any purpose.

FIELD OF THE INVENTION

[0002] The present application relates generally to the detection of biomarkers and the characterization of glucose tolerance, for example, to identify subjects having or likely to have impaired glucose tolerance, which is an indication of pre-diabetes, or diabetes. In various embodiments, the invention relates to one or more biomarkers, methods, devices, reagents, systems, and kits for characterizing impaired glucose tolerance, which may indicate pre-diabetes, and/or diabetes in an individual.

BACKGROUND

[0003] Current methods for determining glucose tolerance include measuring the 2-hour plasma glucose level in individuals participating in an oral glucose tolerance test (OGTT). A typical OGTT consists of the ingestion of a 75-gram oral glucose solution, following an overnight fast, with plasma glucose measurements at baseline ("fasting plasma glucose") and at 2-hours ("2hr-OGTT glucose value") post ingestion. Both fasting plasma glucose and 2-hour OGTT plasma glucose, along with HbA1c values, are currently accepted by the American Diabetes Association for the clinical diagnosis of Type 2 diabetes. Prevention of Type 2 diabetes has primarily been evaluated based on individuals with impaired glucose tolerance, defined as 2-hour OGTT plasma glucose levels ≥ 7.8 mmol/L. *See, e.g.*, Knowler et al. *N. Engl. J. Med.* 2002; 346:393-403 and Tuomilehto et al. *N. Engl. J. Med.* 2001; 344:1343-1350. The development of a proteomic model representative of the OGTT plasma glucose threshold for impaired glucose tolerance, without the need for fasting or glucose administration, , would be highly desirable.

SUMMARY

[0004] In some ~~embodiment~~examples, methods of determining whether a subject has or likely has impaired glucose tolerance are provided. In some ~~embodiment~~examples, methods of identifying subjects with pre-diabetes or likely to develop pre-diabetes are provided. In some ~~embodiment~~examples, methods of identifying subjects likely to develop diabetes are provided.

[0004a] In one aspect, the present disclosure refers to a method of determining whether a subject has impaired glucose tolerance or the likelihood that a subject has impaired glucose tolerance, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least 3 of the N biomarker proteins are selected from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

[0004b] In one aspect, the present disclosure refers to a method of determining whether a subject has pre-diabetes or is likely to develop pre-diabetes or diabetes, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least 3 of the N biomarker proteins are selected from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

[0004c] In one aspect, the present disclosure refers to a method of detecting the levels of N biomarker proteins in a sample, comprising obtaining the sample from the subject, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in the sample from the subject, wherein N is at least 3, and wherein at least 3 of the N biomarker proteins are selected from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

[0005] In some ~~embodiment~~examples, the method herein is for determining whether a subject has impaired glucose tolerance or likely has impaired glucose tolerance, which is an indication of pre-diabetes, or diabetes, comprising obtaining a sample from the subject, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least one of the N biomarker protein is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some ~~embodiment~~examples, N is 3 to 41, N is 4 to 41, N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41, or N is at least 4, or N is at least 5, or N is at least 6, or N is at least 7, or N is at least 8, or N is at least 9, or N is at least 10, or N is at least 11, or N is at least 12, or N is at least 13, or N is at least 14, or N is at least 15, or N is at least 16. In some ~~embodiment~~examples, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41. In some such ~~embodiment~~examples, the subject has impaired glucose tolerance. In some ~~embodiment~~examples, the subject likely has impaired glucose tolerance. In some ~~embodiment~~examples, the subject is likely to develop pre-diabetes. In some ~~embodiment~~examples, the subject is pre-diabetic. In some ~~embodiment~~examples, the subject is likely to develop diabetes. In some ~~embodiment~~examples, the subject has impaired glucose tolerance and has diabetes. In some ~~embodiment~~examples, a subject likely to develop diabetes undertakes preventive measures or undergoes preventive treatment to reduce the likelihood of developing diabetes.

[0006] In some ~~embodiment~~examples, each of the N biomarkers is selected from Table 1. In some ~~embodiment~~examples, at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, and KIN. In some ~~embodiment~~examples, one or two of the N biomarker proteins are INHBC and/or SHBG. In some ~~embodiment~~examples, at least 2 or at least 3 of the N protein biomarkers are selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some ~~embodiment~~examples, at least one of the N biomarker proteins is selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N

biomarker proteins are INHBC, SHBG, and ACY1. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and COL1A1, or two of the N biomarker proteins are SHBG and COL1A1, or wherein three of the N biomarker proteins are INHBC, SHBG, and COL1A1. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and RTN4R, or two of the N biomarker proteins are SHBG and RTN4R, or wherein three of the N biomarker proteins are INHBC, SHBG, and RTN4R. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and CRLF1:CLCF1 complex, or two of the N biomarker proteins are SHBG and CRLF1:CLCF1 complex, or wherein three of the N biomarker proteins are INHBC, SHBG, and CRLF1:CLCF1 complex. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and CBX7, or two of the N biomarker proteins are SHBG and CBX7, or wherein three of the N biomarker proteins are INHBC, SHBG, and CBX7. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and KIN, or two of the N biomarker proteins are SHBG and KIN, or wherein three of the N biomarker proteins are INHBC, SHBG, and KIN. In some ~~embodiment~~examples, N is at least five and five of the N biomarker proteins are INHBC, SHBG, ACY1, COL1A1, and RTN4R. In some ~~embodiment~~examples, N is at least 16, and wherein 16 of the N biomarker proteins are ACY1, COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

[0007] In any of the ~~embodiment~~examples described herein, the subject may be at risk of developing impaired glucose tolerance. In any of the ~~embodiment~~examples described herein, the subject may be at risk of developing pre-diabetes. In any of the ~~embodiment~~examples described herein, the subject may be at risk of developing diabetes. In some ~~embodiment~~examples, the method comprises determining whether the subject has or likely has impaired glucose tolerance, which is an indication of pre-diabetes, or diabetes. In some ~~embodiment~~examples, the method comprises determining whether the subject is pre-diabetic or is likely to develop pre-diabetes or diabetes. In some ~~embodiment~~examples, the diabetes is type 2 diabetes. In some ~~embodiment~~examples, the method comprises administering a treatment to the subject. In some such ~~embodiment~~examples, the treatment comprises administering insulin and/or metformin to the subject. In some ~~embodiment~~examples the treatment comprises implementing a weight loss program, implementing dietary restrictions, implementing caloric restrictions and/or implementing an exercise program for the subject.

[0008] In any of the ~~embodiment~~examples described herein, each of the N biomarker proteins are different from each other. In some ~~embodiment~~examples, the method comprises contacting biomarkers of the sample from the subject with a set of biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker being detected. In some ~~embodiment~~examples, each biomarker capture reagent is an antibody or an aptamer. In some ~~embodiment~~examples, each biomarker capture reagent is an aptamer. In some

~~embodiment~~examples, at least one aptamer is a slow off-rate aptamer. In some ~~embodiment~~examples, at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications. In some ~~embodiment~~examples, each slow off-rate aptamer binds to a biomarker protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

[0009] In any of the ~~embodiment~~examples described herein, the sample may be a blood sample. In any of the ~~embodiment~~examples described herein, the sample may be selected from a serum sample or a plasma sample. In any of the ~~embodiment~~examples described herein, the sample is a plasma sample. In some ~~embodiment~~examples, the subject eats a typical diet before the sample is obtained, wherein the typical diet excludes fasting longer than usual.

[0010] In some ~~embodiment~~examples, a level of at least one biomarker selected from SHBG, COL1A1, CRLF1:CLCF1 complex, FAM20B, COL15A1, KIN, SERPINA11, PELI2, MARCKSL1, CHAD, IL20RB, MYOC, WFDC11, MAP2K4, CALB1, FGFR4, OSTM1, ITIH1, CFAP45, and SFTPD that is higher than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has pre-diabetes, and/or is likely to develop pre-diabetes or diabetes.

[0011] In some ~~embodiment~~examples, a level of at least one biomarker selected from INHBC, ACY1, RTN4R, CBX7, TFF3, HTRA1, FABP12, GAD1, CPM, SVEP1, SOCS7, F9, DLK1, HERC1, STC1, CCL16, SMCO2, GPX2, CCL23, RNASE10, and ZNF134 that is lower than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has pre-diabetes, and/or is likely to develop pre-diabetes or diabetes.

[0012] In some ~~embodiment~~examples, a method described herein is for the purpose of determining a medical insurance premium or life insurance premium. In some ~~embodiment~~examples, a method further comprises determining a medical insurance premium or life insurance premium. In some ~~embodiment~~examples, a method described herein further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

[0013] In some ~~embodiment~~examples, kits are provided. In one aspect, the present disclosure refers to a kit comprising N biomarker protein capture reagents, wherein N is at least 3, and wherein at least 3 of the N biomarker protein capture reagents specifically bind to a biomarker protein selected from COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some ~~embodiment~~examples, a kit comprises N biomarker protein capture reagents, wherein N is at least 3, and wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some ~~embodiment~~examples, N is 3 to 41, N is 4 to 41, N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is

15 to 41, or N is 16 to 41, or N is at least 4, or N is at least 5, or N is at least 6, or N is at least 7, or N is at least 8, or N is at least 9, or N is at least 10, or N is at least 11, or N is at least 12, or N is at least 13, or N is at least 14, or N is at least 15, or N is at least 16. In some ~~embodiment~~examples, N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41. In some such ~~embodiment~~examples, the kit is used to detect the levels of the N biomarker proteins in a sample, wherein the sample is from a subject. In some ~~embodiment~~examples, the kit is used to determine whether the subject has or likely has impaired glucose tolerance, has pre-diabetes, and/or is likely to develop pre-diabetes or diabetes.

[0014] In some ~~embodiment~~examples, each of the N biomarkers is selected from Table 1. In some ~~embodiment~~examples, at least one of the N biomarker proteins is selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, and KIN. In some ~~embodiment~~examples, one or two of the N biomarker proteins are INHBC and/or SHBG. In some ~~embodiment~~examples, at least 2 or at least 3 of the N protein biomarkers are selected from ACY1, COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD. In some ~~embodiment~~examples, at least one of the N biomarker proteins is selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N biomarker proteins are INHBC, SHBG, and ACY1. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and COL1A1, or two of the N biomarker proteins are SHBG and COL1A1, or wherein three of the N biomarker proteins are INHBC, SHBG, and COL1A1. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and RTN4R, or two of the N biomarker proteins are SHBG and RTN4R, or wherein three of the N biomarker proteins are INHBC, SHBG, and RTN4R. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and CRLF1:CLCF1 complex, or two of the N biomarker proteins are SHBG and CRLF1:CLCF1 complex, or wherein three of the N biomarker proteins are INHBC, SHBG, and CRLF1:CLCF1 complex. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and CBX7, or two of the N biomarker proteins are SHBG and CBX7, or wherein three of the N biomarker proteins are INHBC, SHBG, and CBX7. In some ~~embodiment~~examples, two of the N biomarker proteins are INHBC and KIN, or two of the N biomarker proteins are SHBG and KIN, or wherein three of the N biomarker proteins are INHBC, SHBG, and KIN. In some

~~embodiment~~examples, N is at least five and five of the N biomarker proteins are INHBC, SHBG, ACY1, COL1A1, and RTN4R. In some ~~embodiment~~examples, N is at least 16, and wherein 16 of the N biomarker proteins are ACY1, COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

[0015] In some ~~embodiment~~examples, each biomarker capture reagent is an antibody or an aptamer. In some ~~embodiment~~examples, each biomarker capture reagent is an aptamer. In some ~~embodiment~~examples, at least one aptamer is a slow off-rate aptamer.

[0016] In some ~~embodiment~~examples, each slow off-rate aptamer binds to a biomarker protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

[0017] In any of the ~~embodiment~~examples described herein, the sample may be a blood sample. In any of the ~~embodiment~~examples described herein, the sample may be selected from a serum sample and a plasma sample. In some ~~embodiment~~examples, the sample is a plasma sample.

[0018] In any of the ~~embodiment~~examples described herein, each of the N biomarker proteins is different from the other N biomarker proteins. In any of the ~~embodiment~~examples described herein, at least one slow off-rate aptamer may comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications. In some ~~embodiment~~examples, the modifications are hydrophobic modifications. In some ~~embodiment~~examples, the modifications are hydrophobic base modifications. In some ~~embodiment~~examples, one or more of the modifications may be selected from the modifications shown in Figure 2.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **FIG. 1A-C** show the AUC, sensitivity, and specificity of models comprising N biomarker proteins, as described in Example 4. FIG. 1A shows results for models in which one biomarker protein is added to the model at a time, as described in Example 4. FIG. 1B shows results for models in which one biomarker protein is removed from the model at a time, as described in Example 4. FIG. 1C shows results for models that comprise randomly selected N biomarkers from Table 1, as described in Example 4.

[0020] **FIG. 2** shows certain nucleobase modifications that can be used in an aptamer.

[0021] **FIG. 3** shows a nonlimiting exemplary computer system for use with various computer-implemented methods described herein.

[0022] **FIG. 4** shows glucose tolerance status as determined by the method described in Example 2 (y-axis) relative to the classification determined by a traditional OGTT (x-axis).

CLAIMS

1. A method of determining whether a subject has impaired glucose tolerance or the likelihood that a subject has impaired glucose tolerance, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least ~~one~~ 3 of the N biomarker proteins ~~is~~ are selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

2. A method of determining whether a subject has pre-diabetes or is likely to develop pre-diabetes or diabetes, comprising forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in a sample from the subject, wherein N is at least 3, and wherein at least ~~one~~ 3 of the N biomarker proteins ~~is~~ are selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

3. A method of detecting the levels of N biomarker proteins in a sample, comprising obtaining the sample from the subject, forming a biomarker panel having N biomarker proteins, and detecting the level of each of the N biomarker proteins in the sample from the subject, wherein N is at least 3, and wherein at least ~~one~~ 3 of the N biomarker proteins ~~is~~ are selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

4. The method of any one of the preceding claims, wherein N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41.

5. The method of any one of the preceding claims, wherein N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N

is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41.

6. The method of any one of the preceding claims, wherein each of the N biomarker proteins is selected from Table 1.

7. The method of any one of the preceding claims, wherein at least one of the N biomarker proteins is selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, ~~and~~ KIN, and ACY1.

8. The method of any one of the preceding claims, wherein one or two of the N biomarker proteins are INHBC and/or SHBG.

9. The method of any one of the preceding claims, wherein ~~at least 2, at least 3,~~ at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N protein biomarkers are selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

10. The method of any one of the preceding claims, wherein at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 of the N biomarker proteins is selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.

11. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and ACY1, or two of the N biomarker proteins are SHBG and ACY1, or three of the N biomarker proteins are INHBC, SHBG, and ACY1.

12. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and COL1A1, or two of the N biomarker proteins are SHBG and COL1A1, or wherein three of the N biomarker proteins are INHBC, SHBG, and COL1A1.

13. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and RTN4R, or two of the N biomarker proteins are SHBG and RTN4R, or wherein three of the N biomarker proteins are INHBC, SHBG, and RTN4R.

14. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CRLF1:CLCF1 complex, or two of the N biomarker proteins are SHBG and CRLF1:CLCF1 complex, or wherein three of the N biomarker proteins are INHBC, SHBG, and CRLF1:CLCF1 complex.

15. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and CBX7, or two of the N biomarker proteins are SHBG and CBX7, or wherein three of the N biomarker proteins are INHBC, SHBG, and CBX7.

16. The method of any one of the preceding claims, wherein two of the N biomarker proteins are INHBC and KIN, or two of the N biomarker proteins are SHBG and KIN, or wherein three of the N biomarker proteins are INHBC, SHBG, and KIN.

17. The method of any one of the preceding claims, wherein N is at least five and five of the N biomarker proteins are INHBC, SHBG, ~~ACY1,~~ COL1A1, ~~and~~ RTN4R, and ACY1.

18. The method of any one of the preceding claims, wherein N is at least 16, and wherein 16 of the N biomarker proteins are ~~ACY1,~~ COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, ACY1, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

19. The method of any one of the preceding claims, wherein the sample is a blood sample, a plasma sample, or a serum sample.

20. The method of any one of the preceding claims, wherein the subject continues eating a typical diet and a glucose solution is not administered to the subject before the sample is obtained.

21. The method of any one of the preceding claims, wherein a level of at least one biomarker selected from SHBG, COL1A1, CRLF1:CLCF1 complex, FAM20B, COL15A1, KIN, SERPINA11, PELI2, MARCKSL1, CHAD, IL20RB, MYOC, WFDC11, MAP2K4, CALB1, FGFR4, OSTM1, ITIH1, CFAP45, and SFTPD that is higher than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.

22. The method of any one of the preceding claims, wherein a level of at least one biomarker selected from INHBC, ~~ACY1,~~ RTN4R, CBX7, TFF3, HTRA1, FABP12, GAD1, CPM, SVEP1, SOCS7, F9, ACY1, DLK1, HERC1, STC1, CCL16, SMCO2, GPX2, CCL23, RNASE10, and ZNF134 that is lower than a control level of the respective biomarker, indicates that the subject has or likely has impaired glucose tolerance, has or is likely to develop pre-diabetes, and/or is likely to develop diabetes.

23. The method of any one of the preceding claims, wherein the subject is at risk of developing diabetes.

24. The method of any one of the preceding claims, comprising determining whether the subject has or likely has impaired glucose tolerance.

25. The method of claim 24, wherein the subject likely has impaired glucose tolerance.

26. The method of claim 24, wherein the subject has impaired glucose tolerance.

27. The method of any one of claims 2 to 26, comprising determining whether the subject is likely to develop or has pre-diabetes.

28. The method of claim 27, wherein the subject is likely to develop pre-diabetes.

29. The method of claim 27, wherein the subject has pre-diabetes.

30. The method of any one of claims 25, 26, 28, and 29, wherein the subject is likely to develop diabetes.

31. The method of claim 30, wherein the diabetes is type 2 diabetes.

32. The method of any one of claims 21-23, 25, 26, and 28-31, comprising administering a treatment to the subject.

33. The method of claim 32, wherein the treatment comprises insulin, metformin, implementing a weight loss program, implementing dietary restrictions, implementing caloric restrictions and/or implementing an exercise program.

34. The method of any one of the preceding claims, wherein the method comprises contacting biomarker proteins of the sample or samples with a set of biomarker capture reagents, wherein each biomarker capture reagent of the set of biomarker capture reagents specifically binds to a different biomarker protein being detected.

35. The method of claim 34, wherein each biomarker capture reagent is an antibody or an aptamer.

36. The method of claim 35, wherein each biomarker capture reagent is an aptamer.

37. The method of claim 36, wherein at least one aptamer is a slow off-rate aptamer.

38. The method of claim 37, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

39. The method of claim 37 or claim 38, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

40. The method of any one of the preceding claims, wherein if the subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes or diabetes, the subject is recommended a regimen comprising weight loss, blood sugar control, and/or drug treatment.

41. The method of any one of the preceding claims, wherein the determining comprises analyzing the levels of the N biomarker protein levels using a classification model or an elastic net logistic regression model.

42. The method of any one of the preceding claims, wherein the method comprises determining whether a subject has or likely has impaired glucose tolerance, has pre-diabetes, or is likely to develop pre-diabetes or diabetes for the purpose of determining a medical insurance premium or life insurance premium.

43. The method of any one of the preceding claims, wherein the method further comprises determining a medical insurance premium or life insurance premium.

44. The method of any one of the preceding claims, wherein the method further comprises using information resulting from the method to predict and/or manage the utilization of medical resources.

45. A kit comprising N biomarker protein capture reagents, wherein N is at least 3, and wherein at least ~~one~~ 3 of the N biomarker protein capture reagents specifically bind~~s~~ to a biomarker protein selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

46. The kit of claim 45, wherein N is 3 to 41, or N is 4 to 41, or N is 5 to 41, or N is 6 to 41, or N is 7 to 41, or N is 8 to 41, or N is 9 to 41, or N is 10 to 41, or N is 11 to 41, or N is 12 to 41, or N is 13 to 41, or N is 14 to 41, or N is 15 to 41, or N is 16 to 41.

47. The kit claim 45 or 46, wherein N is 3, or N is 4, or N is 5, or N is 6, or N is 7, or N is 8, or N is 9, or N is 10, or N is 11, or N is 12, or N is 13, or N is 14, or N is 15, or N is 16, or N is 17, or N is 18, or N is 19, or N is 20, or N is 21, or N is 22, or N is 23, or N is 24, or N is 25, or N is 26, or N is 27, or N is 28, or N is 29, or N is 30, or N is 31, or N is 32, or N is 33, or N is 34, or N is 35, or N is 36, or N is 37, or N is 38, or N is 39, or N is 40, or N is 41.

48. The kit of any one of claims 45 to 47, wherein each of the N biomarker protein capture reagents specifically binds to a different biomarker protein.

49. The kit of any one of claims 45 to 48, wherein each of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from Table 1.

50. The kit of any one of claims 45 to 49, wherein at least one of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, ~~and~~ KIN, and ACY1.

51. The kit of any one of claims 45 to 50, wherein one or two of the N biomarker protein capture reagents specifically binds to INHBC and/or SHBG.

52. The kit of any one of claims 45 to 51, wherein ~~at least 2 or~~ at least ~~3~~4 of the N protein biomarker capture reagents each specifically bind to a protein selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1:CLCF1 complex, CBX7, KIN, SERPINA11, PELI2, TFF3, FABP12, GAD1, SVEP1, SOCS7, F9, ACY1, STC1, MYOC, WFDC11, CALB1, CCL16, SMCO2, CCL23, OSTM1, RNASE10, ITIH1, ZNF134, CFAP45, and SFTPD.

53. The kit of any one of claims 45 to 52, wherein at least one of the N biomarker protein capture reagents specifically binds to a protein selected from FAM20B, COL15A1, MARCKSL1, HTRA1, CHAD, CPM, DLK1, HERC1, IL20RB, MAP2K4, GPX2, and FGFR4.

54. The kit of any one of claims 45 to 53, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or ACY1, or each of two of the N biomarker protein capture reagents specifically binds to SHBG or ACY1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and ACY1.

55. The kit of any one of claims 45 to 54, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or COL1A1, each of two of the N biomarker protein capture reagents specifically binds to SHBG or COL1A1, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and COL1A1.

56. The kit of any one of claims 45 to 55, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or RTN4R, each of two of the N biomarker protein capture reagents specifically binds to SHBG or RTN4R, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and RTN4R.

57. The kit of any one of claims 45 to 56, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CRLF1:CLCF1 complex, each of two of the N biomarker protein capture reagents specifically binds to SHBG or

CRLF1:CLCF1 complex, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and CRLF1:CLCF1 complex.

58. The kit of any one of claims 45 to 57, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or CBX7, each of two of the N biomarker protein capture reagents specifically binds to SHBG or CBX7, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and CBX7.

59. The kit of any one of claims 45 to 58, wherein each of two of the N biomarker protein capture reagents specifically binds to INHBC or KIN, each of two of the N biomarker protein capture reagents specifically binds to SHBG or KIN, or each of three of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, and KIN.

60. The kit of any one of claims 45 to 59, wherein N is at least five and each of five of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from INHBC, SHBG, ~~ACY1,~~ COL1A1, ~~and~~ RTN4R, and ACY1.

61. The kit of any one of claims 45 to 60, wherein N is at least 16, and wherein each of 16 of the N biomarker protein capture reagents specifically binds to a biomarker protein selected from ~~ACY1,~~ COL1A1, RTN4R, CRLF1, CBX7, KIN, SERPINA, PELI2, TFF3, FABP12, ACY1, INHBC, SHBG, FAM20B, COL15A1, MARCKSL1, and HTRA1.

62. The kit of any one of claims 45 to 61, wherein each of the N biomarker capture reagents is an antibody or an aptamer.

63. The kit of claim 62, wherein each biomarker capture reagent is an aptamer.

64. The kit of claim 63, wherein at least one aptamer is a slow off-rate aptamer.

65. The kit of claim 64, wherein at least one slow off-rate aptamer comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least 10 nucleotides with modifications.

66. The kit of claim 64 or claim 65, wherein each slow off-rate aptamer binds to its target protein with an off rate ($t_{½}$) of ≥ 30 minutes, ≥ 60 minutes, ≥ 90 minutes, ≥ 120 minutes, ≥ 150 minutes, ≥ 180 minutes, ≥ 210 minutes, or ≥ 240 minutes.

67. The kit of any one of claims 45 to 66, for use in detecting the N biomarker proteins in a sample from a subject.

68. The kit of claim 67, for use in determining whether the subject has or likely has impaired glucose tolerance or has pre-diabetes, or is likely to develop pre-diabetes or diabetes.