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(54) **Title:** DYNAMIC STRESS FACTOR FOR USE IN DIAGNOSTIC AND PROGNOSTIC METHODS

(57) **Abstract:** The invention relates to methods wherein an index of blood glucose volatility is determined, and the use of the determined index for diagnostic and prognostic purposes. In exemplary aspects, the index of blood glucose volatility is used in diagnostic, prognostic, therapeutic, and screening methods relating to diabetes, severe hypoglycemic episodes, and long term complications of diabetes. Related methods implemented by a processor in a computer, computer-readable storage media, and systems are further provided herein.



DYNAMIC STRESS FACTOR FOR USE IN DIAGNOSTIC AND PROGNOSTIC METHODS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/568,285, filed on December 8, 2011, the contents of which are incorporated by reference in its entirety.

STATEMENT OF GOVERNMENT RIGHTS

[0002] This invention was made with government support under National Institutes of Health Grant No. 1R01HL 102334-01 and National Science Foundation - Division of Mathematical Sciences Grant No. 0634590. The government has certain rights in this invention.

BACKGROUND

[0003] Emerging evidence suggests that chronic wide glucose fluctuations increase the risk of severe hypoglycemia and may be instrumental for the development of chronic diabetes complications. Therefore, development of sensitive tools to analyze blood glucose (BG) fluctuations and guide appropriate therapeutic changes to blunt wide BG excursions will have a critical role in preventing acute and chronic complications and improve quality of life in diabetic patients. Continuous glucose monitors (CGMs) that record glucose levels at short, regular intervals throughout the day afford patients and physicians the flexibility to track glucose trends, evaluate frequency and severity of hypoglycemia including during nocturnal patterns, and assess individualized response to exercise and various other stressors. As the use of CGMs increases, conclusions previously drawn from single monitor blood glucose (SMBG) data can be tested against this more robust data set to guide optimization in individualized insulin regimens, to effectively prevent severe hypoglycemia, to blunt hyperglycemic peaks in response to meals and other stressors, and to evaluate the longer term consequences of glucose variability on the development of diabetic complications. Traditionally, HbA_{1c} is used to assess glycemic control and risk of complications in patients with diabetes [1,2]. However, emerging evidence suggests that glucose variability may also play an important role in assessing the risk for hypoglycemia and/or in the development of microvascular complications and cardiovascular disease [3-5] via several mechanisms including its role in oxidative stress and vascular pathology [6-8]. Several metrics to quantify glucose variability have been employed to date [9-12]. However, until

recently the only data available for such studies were obtained through five- or seven-point single monitor profiles that provided only a restricted view of a patient's glucose dynamics over 24 hours [1,13]. These discrete glucose measurements are limiting in both the amount of information available for the analysis of glycemic variability and the methods by which variability can be examined. Metrics currently used to quantify glucose variability include, but are not limited to, standard deviation (SD), mean amplitude of glycemic excursions (MAGE) [14], mean of daily differences (MODD) [15], and continuous overall net glycemic action (CONGA(n)) [16], and more recently standard deviation rate of change (SDRC), average absolute rate of change (AARC) [17], glucose error grid analysis (CGEGA), and prediction-error grid analysis (PRED-EGA) [18]. None of these, however, fully address the issue of glucose volatility in the context of hypoglycemia. These metrics are furthermore limited in that they look at only the general trends of blood glucose levels for only short periods of time, and do not account for all types and sizes of glycemic transitions.

SUMMARY

[0004] The invention relates to diagnostic, prognostic, and therapeutic methods wherein an index of blood glucose volatility is determined. In exemplary aspects, the methods relate to diabetes, severe hypoglycemic episodes, and long term complications of diabetes.

[0005] The invention provides a method of determining a subject's risk for a severe hypoglycemic episode. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to be at risk for a severe hypoglycemic episode.

[0006] The invention also provides a method of determining a subject's need for prophylaxis of a severe hypoglycemic episode. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to need prophylaxis of a severe hypoglycemic episode.

[0007] The invention further provides a method of preventing a severe hypoglycemic episode in a subject. In exemplary embodiments, the method comprises the steps of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject prophylaxis

for a severe hypoglycemic episode in an amount sufficient to prevent the severe hypoglycemic episode, when the index of blood glucose volatility is decreased, as compared to a control index.

[0008] The invention also provides a method of reducing a subject's risk for a severe hypoglycemic episode in a subject. In exemplary embodiments, the method comprises the step of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject a therapeutic agent for the treatment of diabetes, or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0009] The invention provides a method of monitoring a subject's risk for a severe hypoglycemic episode. In exemplary embodiments, the method comprises the steps of determining a first index of blood glucose volatility of the subject at a first time point and determining a second index of blood glucose volatility of the subject at a second time point, wherein the first time point occurs before the second time point, wherein the subject's risk for a severe hypoglycemic episode is increased, when the second index is less than the first index.

[0010] The invention further provides a method of determining the efficacy of a compound for reducing blood glucose volatility in a subject. In exemplary embodiments, the method comprises the steps of (i) determining a first index of blood glucose volatility of the subject before administration of the compound and (ii) determining a second index of blood glucose volatility of the subject after administration of the compound, wherein the compound is determined as effective for reducing blood glucose volatility, when the second index is higher than the first index.

[0011] Furthermore, the invention provides a method of determining a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control, the subject is determined to be at risk for a long term complication of diabetes.

[0012] Moreover, the invention provides a method of determining a subject's need for prophylaxis of a long term complication of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the

index of blood glucose volatility is decreased, as compared to a control, the subject is determined to need prophylaxis of a long term complication of diabetes.

[0013] The invention additionally provides a method of preventing a long term complication of diabetes in a subject. In exemplary embodiments, the method comprises the steps of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject a therapeutic agent for the treatment of diabetes or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0014] The invention also provides a method of reducing a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the step of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject a therapeutic agent for the treatment of diabetes, or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0015] The invention provides a method of monitoring a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the steps of determining a first index of blood glucose volatility of the subject at a first time point and determining a second index of blood glucose volatility of the subject at a second time point, wherein the subject's risk for a long term complication of diabetes is increased, when the second index is less than the first index.

[0016] In exemplary aspects, the index of blood glucose volatility is determined by analyzing blood glucose level data of the subject collected and recorded at short, regular intervals. The data are analyzed for the frequency at which a glycemic transition of a given magnitude occurs within a given time period. The glycemic transition considered in the calculation of the index of blood glucose volatility is at least or about a minimum magnitude threshold, $MT_{m_{th}}$. In the calculation of the index of blood glucose volatility, the frequency at which a glycemic transition of a given magnitude occurs within a given time period is multiplied by the magnitude to obtain a weighted value W_M . A W_M is determined for each magnitude M for which the absolute value is greater than zero. In the index of blood glucose volatility, each W_M determined for each magnitude M for which the absolute value is greater than zero is summed.

[0017] In some aspects, determining the index of blood glucose volatility comprises the steps of (a) analyzing blood glucose level data of the subject collected and recorded at short, regular intervals, wherein the data are analyzed for an occurrence of a glycemic transition which is at least or about a minimum magnitude threshold, MT_{min} , (b) determining a magnitude, M , of each glycemic transition which is equivalent to or greater than MT_{min} , wherein M is a positive integer, when the glycemic transition is an increase, and M is a negative integer, when the glycemic transition is a decrease, wherein M is determined by counting the number of magnitude thresholds (MT) a glycemic transition meets, wherein each MT , MT_n , is fixed at a value that equals n multiplied by MT_{min} ; (c) for each unique M having an absolute value greater than 1, calculating a value W_M by multiplying the value M by the number of occurrences of glycemic transitions within a total time period, T , having that M , and (d) summing each W_M for each M to obtain an index of glucose volatility.

[0018] In exemplary aspects, the index of blood glucose volatility is determined by (a) analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined, (b) determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1.

[0019] In some aspects, determining the index of blood glucose volatility comprises the steps of: (a) analyzing blood glucose level data of the subject for an occurrence of a glycemic transition which is at least or about a minimum magnitude threshold, MT_{min} , wherein MT^{TM} is a number between 10 and 60; (b) determining the magnitude, M , of each glycemic transition which is equivalent to or greater than MT^{TM} , wherein M is a positive integer, when the glycemic transition is an increase, and M is a negative integer, when the glycemic transition is a decrease, wherein M is determined by counting the number of magnitude thresholds (MT) a glycemic transition meets, wherein each MT , MT_n , is fixed at a value that equals n multiplied by MT_{min} ; (c) for each occurrence of a glycemic transition having a determined M , assigning based on the

M of the glycemic transition the occurrence to one of a series of magnitude bins, wherein each magnitude bin of the series has a unique M, (d) determining the number of occurrences within each magnitude bin; (e) determining the duration, D, of each glycemic transition having a determined M, (f) for each occurrence of a glycemic transition having a determined D, assigning based on the D of the glycemic transition the occurrence to one of a series of duration bins, wherein each duration bin of the series encompasses a unique range of D values, wherein an occurrence is assigned to the duration bin having the range of D values within which the D of the occurrence falls, (g) for each magnitude bin, multiplying the magnitude, M, of the bin by the number of occurrences of glycemic transitions in that magnitude bin, thereby obtaining a number, W_M , (h) summing each W_M to obtain an index of glucose volatility, wherein the blood glucose level data was collected and recorded at short, regular intervals.

[0020] The invention further provides a method implemented by a processor in a computer. In exemplary embodiments, the method comprises (i) receiving a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_f , each data point comprising a blood glucose measurement value and a time value; (ii) determining, via the processor, a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing; (iii) determining, via the processor, for each pair of successive transition points, $(X_{1,i}, X_{2,i})$: (1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ; (2) an amount, t_i , of time elapsed between the pair of transition points; and (3) whether the blood glucose level is increasing or decreasing between the pair of transition points; (iv) determining, via the processor, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{max}}$ and (ii) a value Y_i equal to Z ; (v) determining, via the processor, an average daily frequency, F_Z , of changes of each magnitude Z ; and (vi) determining, via the processor, a weighted sum, $DySF$, of the magnitude of large transitions.

[0021] The invention further more provides a computer-readable storage medium having stored thereon machine-readable instructions executable by a processor. In exemplary embodiments, the computer-readable storage medium comprises:

instructions for causing the processor to receive a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_p , each data point comprising a blood glucose measurement value and a time value;

instructions for causing the processor to determine a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing;

instructions for causing the processor to determine, for each pair of successive transition points, $(X_{1,i}, X_{2,i})$:

(1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ;

(2) an amount, t_i , of time elapsed between the pair of transition points; and

(3) whether the blood glucose level is increasing or decreasing between the pair of transition points;

instructions for causing the processor to determine, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{\max}}$ and (ii) a value Y_i equal to Z ;

instructions for causing the processor to determine an average daily frequency, F_Z , of changes of each magnitude Z ; and

instructions for causing the processor to determine a weighted sum, $DySF$, of the magnitude of large transitions.

[0022] The invention moreover provides a system comprising a processor and a memory device coupled to the processor. In exemplary embodiments, the memory device storing machine readable instructions, when executed by the processor, cause the processor to:

receive a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_p , each data point comprising a blood glucose measurement value and a time value;

determine a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing;

determine, for each pair of successive transition points, $(X_{1,i}, X_{2,i})$:

(1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ;

(2) an amount, t_i , of time elapsed between the pair of transition points; and

(3) whether the blood glucose level is increasing or decreasing between the pair of transition points;

determine, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{\max}}$ and (ii) a value Y_i equal to Z ;

determine an average daily frequency, F_Z , of changes of each magnitude Z ; and

determine a weighted sum, $DySF$, of the magnitude of large transitions.

[0023] The invention additionally provides a method of assessing an index of blood glucose volatility of a subject. The method comprises the steps of (a) analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, $MT_{m_{\min}}$, an F_M is determined, (b) determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1. , wherein steps (a) through (c) are determined by the aforementioned system.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figure 1 collectively illustrates the calculation of $DySF$ through transition density profiles. Figure 1A) is a graph of patient input glucose data, illustrating the 40 mg/dL increments in alternating grey and white. Figure 1B) is a graph of smoothed input data in bins. Figure 1C) is a graph of a blow-up of data in (Figure 1B) highlighting the monotonic changes to be recorded in the Transition Density Profile (Figure 1D) which compiles monotonic changes that occur in a specific time interval. The shaded region shows all the monotonic transitions that occurred in less than 1 h. $DySF$ is calculated as the sum of the number of transitions greater than one, weighted by the magnitude of the transition. (Example above: $DySF = 0.3 * |-7| + 0.3 * |-3| +$

$2.6*|-2| + 1.75*|2| + 0.9*|3| + 0.9*|4| + 0.3 *|5| = 19.5$. The weights listed as 0.3 and 2.6 for example are determined by the number of actual transitions occurred divided by the length of time the sample set is measured. Since there was one transition of -7 bins that occurred during the 5000 minutes or 3.4 days measured, we get $1/3.4 = 0.3$ to be our weighted factor for this transition.)

[0025] Figure 2 collectively illustrates a dashboard of Glycemic Variability, Logistic Regression P-values, and Correlations of Previous Metrics to DySF. Figure 2A) illustrates the Glycemic Variability Profile for one patient with type 1 diabetes created by the CGM-GUIDE software [18]. Provides user adjustable bin thresholds, transition density profiles, statistics (mean, SD, glycemic times/areas), and metric calculations including DySF, CONGA, MODD and MAGE. Figure 2B is a table of p-values from individual logistic regressions to predict the number of hypoglycemic episodes (HE) based on the described metrics. DySF shows the most predictive power among metrics in children 8-14 yr (p-value= 0.018). Figure 2C is a correlation of previous statistics and metrics to DySF. Confidence intervals were obtained as the central 95% of correlation coefficients observed on the basis of 10,000 bootstrap samples (of size equal to the original sample). Dots represent the means of the resulting empirical distributions and are essentially equivalent to the one sample point estimates from the original data.

[0026] Figure 3 demonstrates that DySF Distinguishes Patients into Visually Distinct Classes Independent of HbA1c Level. Figures 3A and 3B illustrate two individuals within the same HbA1c level (< 7) whose volatility is separated by DySF. Figures 3C-D illustrate two individuals within the same HbA1c level (> 7) whose volatility is separated by DySF.

[0027] Figure 4 is essentially the same information presented in Figures 1B and 1D.

[0028] Figure 5 is a table demonstrating Integrated Glycemic Variability Assessment for a Diabetic versus Non-diabetic controls. Metric calculations for a representative T1D patient (Patient3) verses average non-diabetic reference values. Non-diabetic reference values for mean glucose, percent time hyperglycemic and percent time hypoglycemic were obtained from McDonnell et al.'s study on 10 healthy controls. Non-diabetic reference values for the remaining metrics were obtained from Cameron et al.'s study on 12 healthy, non-diabetic controls.

[0029] Figure 6 is a table demonstrating Standard measures of glucose variability. BG = Blood glucose reading at time t minutes after start of observations and t_i = time in minutes after start of observations when the i^{th} observation is taken.

[0030] Figure 7 demonstrates the *Transition Density Profile*: a cumulative measure of severity and frequency of glycemic change. Figure 7A is a graph of Raw CGM data from a diabetic patient with mean glucose equal to 121.89 mg/dL. Colored horizontal bands indicate different user-defined glucose ranges. Figure 7B is a graph of raw CGM data is categorized into bins according to user-defined threshold ranges, defined here as (0, 50, 70, 180, 220, 300 mg/dL). The percent time spent in each bin is displayed in red. Figure 7C is a graph of the Area under the curve (AuC) above and below the hyper-/hypoglycemic limit of 180 (red) and 70 (blue) mg/dL, respectively. AuC is a measure of hyper- and hypoglycemic severity in conjunction with the (Figure 7D) transition speed, or the rate of change in blood glucose at the transition between each threshold. (Thresholds are defined as 0, 40, 70, 120, 180, 220, and 300.) (insert) Histogram of the slopes over every 5-minute time interval. Figure 7E is a graph of an Example of binned CGM data where a monotonic increase and decrease was observed. The duration of these monotonic increases/decreases was then calculated for the (Figure 7F) *transition density profile*. The number of transitions per day where transitions are described as the magnitude of every continuous monotonic change in blood glucose levels, after smoothing, sorted into the number of thresholds crossed (e.g. 2, 4, -3, -5) and separated into the time interval necessary to complete each change (e.g. < 1 h, between 1-2 h, 2-3 h, etc.). Negative numbers indicate monotonic decreases.

[0031] Figure 8 demonstrates the CGM-GUIDE Interface. CGM-GUIDE allows for user-defined input of the threshold ranges, the hyper- and hypoglycemic limits, and the CONGA 'n' value. Glucose variability metrics (SD, MODD, CONGA(n), and MAGE) are calculated as described in Methods in conjunction with glycemic statistics (time spent within thresholds, time spent in hyperglycemic / hypoglycemic conditions, area under the curve, and mean glucose). Displays for area under the curve, transition speed, and slope histogram plots (Fig. 1C-D) are available through a plot menu option.

[0032] Figure 9 is a block diagram depicting a system for calculating and/or monitoring DySF.

[0033] Figure 10 is a block diagram depicting a device operable to calculate and/or monitor DySF.

[0034] Figure 11 is a flow chart depicting an exemplary method for computing DySF.

[0035] Figure 12 is a graph depicting an exemplary set of CGM data.

[0036] Figure 13 is a graph of raw glucose data. Glucose level (mg/dL) vs. time (min) and Bins 1-6 are shown.

[0037] Figure 14 is a graph of binned glucose data. Bins 1-6 vs. time (min).

[0038] Figure 15 is a graph of binned glucose data. Bins 1-6 vs. time (min). Monotonic transitions are marked with vertical lines and the monotonic transition time intervals are labeled below.

[0039] Figure 16 is a graph of the binned glucose data. Bins 1-6 vs. time (min). Monotonic transitions are marked with vertical lines, the monotonic transition time intervals, as well as the magnitude, are labeled below each transition.

[0040] Figure 17 is a graph similar to Figure 16, but demonstrating the criteria for a "relevant" transition and the calculation of DySF.

[0041] Figure 18 is a graph of binned glucose data wherein data is missing and there is a gap in the data and how gaps are treated.

[0042] Figure 19 is a graph similar to that of Figure 18 but with vertical lines marking the glycemic transitions, the magnitude of each glycemic transition (wherein each has a duration time within 1 hour) and a gap time of 5 minutes or 25 minutes and the calculation of DySF.

[0043] Figure 20 is a graph of Factors 1 to 3 and 17 different metrics as described in Example 5.

DETAILED DESCRIPTION

[0044] *Index of Glucose Volatility*

[0045] The methods described herein comprise determining an index of blood glucose volatility of the subject. As used herein, the term "glucose volatility" is synonymous with "glucose variability," "glycemic volatility," and "glycemic variability." In exemplary aspects,

the index of blood glucose volatility is determined through any one of the ways presented herein. In exemplary aspects, the index of blood glucose volatility is dynamic stress factor (DySF), as further described herein. In exemplary aspects, the index of blood glucose volatility is determined through use of the system, method implemented by a processor in a computer, or computer readable storage medium of the invention.

[0046] *Blood Glucose Data*

[0047] The methods of the present disclosures comprises determining an index of glucose volatility based on blood glucose data of a subject. The data evaluated are blood glucose level data of a subject collected and recorded at short, regular intervals. In exemplary embodiments, the data are blood glucose level data of a subject collected and recorded at intervals X minutes apart, wherein X is a number between 0.5 and 30 (e.g., at 30 second intervals, 1 minute intervals, 2 minute intervals, 3 minute intervals, 4 minute intervals, or 5 minute intervals). In exemplary embodiments, the data are blood glucose level data of a subject collected and recorded at X minute intervals, wherein X is a number between 5 and 30 (5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30). In exemplary aspects, X is a number between about 5 and about 29, or between about 5 and about 28, or between about 5 and about 27, or between about 5 and about 26, or between about 5 and about 25, or between about 5 and about 24, or about 5 and about 23, or between about 5 and about 22, or between about 5 and about 21 or about 5 and about 20, or between about 5 and about 19, or between about 5 and about 18, or between about 5 and about 17, or between about 5 and about 16, or between about 5 and about 15, or between about 5 and about 14, or between 5 and about 13, or between about 5 and about 12, or between about 5 and about 11, or between about 5 and 10. In exemplary aspects, X is a number between about 6 and about 15, or between about 7 and about 15, or between about 8 and about 15, or between about 9 and about 15, or between about 10 and about 15, or between about 11 and about 15, or between about 12 and about 15, or 13, or 14. In exemplary aspects, X is a number between about 6 and about 20, or between about 7 and about 20, or between about 8 and about 20, or between about 9 and about 20, or between about 10 and about 20, or between about 11 and about 20, or between about 12 and about 20, or between about 13 and about 20, or between about 14 and about 20, or between about 15 and about 20.

[0048] *Sources of Blood Glucose Data*

[0049] In exemplary aspects, the blood glucose data on which the index of glucose volatility is determined are raw or unprocessed data collected and recorded from a continuous glucose monitor (CGM), or like device. The data may be recorded and collected on Medtronic's Minimed Paradigm RTS with a subcutaneous probe attached to a small transmitter that sends interstitial glucose levels to a small pager sized receiver, or a DexCom STS System which is a hypodermic probe with a small transmitter, or a FreeStyle Navigator commercially available from Abbott Laboratories. Alternatively, the blood glucose data may be data collected and recorded via non invasive methods, e.g., methods which utilize infrared or near-infrared light, electric current, or ultrasound for measuring blood glucose levels. The blood glucose data may be data collected and recorded via a small transdermal patch that can wirelessly monitor blood glucose levels. Such patches are being developed through companies, such as, Sano Intelligence (San Francisco, CA), and are also described in International Patent Application Publication No. WO2001/091626 and U.S. Patents 6,503,198; 6,475,425, and 6,952,263.

[0050] Alternatively, the blood glucose data may be data collected and recorded from a standard glucose meter which determines the approximate concentration of glucose in the blood. The glucose meter may be one that works with test strips or discs containing chemicals (e.g., glucose oxidase) that react with glucose in a drop of blood. The drop of blood may be from a finger prick or an alternative site on the body, e.g., forearm. In exemplary aspects, the glucose meter records the blood glucose level and retains this information for better diabetes management. The glucose meter in some aspects comprises software for recording and maintaining the collected blood glucose data. The glucose meter in exemplary aspects comprises insulin injection devices, personal digital assistants (PDA), cellular transmitters, clocks, memory, alarms, and/or a radio transmitter to an insulin pump.

[0051] In exemplary aspects, the blood glucose data on which the index of glucose volatility is determined are processed data, manipulated data, data which has been normalized or smoothed relative to the raw data, e.g., raw data from a CGM or like device. In exemplary aspects, the data from the CGM or like device is normalized or smoothed through use of a computer software program called CGM-GUIDE, which is further described herein in Example 4.

[0052] *Glycemic Transitions*

[0053] The methods of the present disclosures comprises determining an index of glucose volatility, wherein the index is determined by evaluating blood glucose data for occurrences of glycemic transitions. As used herein, the term "glycemic transition" refers to a change (either an increase or decrease) in blood glucose level occurring between two time points, wherein the change in blood glucose occurs without any intervening change of the opposite type. For example, a glycemic transition may be an increase in blood glucose levels between two time points, t_1 and t_2 , wherein the increase occurs without any decrease in blood glucose levels between t_1 and t_2 . For example, a glycemic transition may be a decrease in blood glucose levels between two time points, t_1 and t_2 , wherein the decrease occurs without any increase in blood glucose levels between t_1 and t_2 . Each glycemic transition may be characterized by its magnitude. The absolute magnitude of a glycemic transition occurring between t_1 and t_2 is determined by taking the absolute value of the difference between the blood glucose level at t_1 and the blood glucose level at t_2 .

[0054] In exemplary aspects, the index of glucose volatility is determined by evaluating data for occurrences of glycemic transitions that meet a minimum magnitude threshold, MT_{min} . In exemplary embodiments, MT^{TM} is a number between 10 and 60 (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60). In exemplary aspects, MT_{min} is a number within 10 and 40, 15 and 55 or 20 and 50, or 25 and 45.

[0055] For each glycemic transition which is equivalent to or greater than MT_{min} , a magnitude, M , is determined and/or assigned. M is a positive integer, when the glycemic transition is an increase (e.g., when the blood glucose level at t_1 is smaller than the blood glucose level at t_2). M is a negative integer, when the glycemic transition is a decrease (e.g., when the blood glucose level at t_1 is larger than the blood glucose level at t_2). M is determined by counting the number of magnitude thresholds (MT) a glycemic transition crosses, wherein each MT_n is fixed at a value that equals n multiplied by MT_{min} . For example, if MT^{TM} is 40 mg/dL, then MT_1 is 40 mg/dl, MT_2 is 80 mg/dl, MT_3 is 120 mg/dl, MT_4 is 160 mg/dl, MT_5 is 200 mg/dl, MT_6 is 240 mg/dl, and MT_7 is 280 mg/dl, MT_{-1} is 40 mg/dl, MT_{-2} is 80 mg/dl, MT_{-3} is 120 mg/dl, MT_{-4} is 160 mg/dl, MT_{-5} is 200 mg/dl, MT_{-6} is 240 mg/dl, and MT_{-7} is 280 mg/dl. If a glycemic transition is one in which the blood glucose level is 30 mg/dl at t_1 and the blood glucose level at t_2 is 90 mg/dl, then the M for this glycemic transition is 2, because transitioning from 30 mg/dl to 90 mg/dl

crosses MT_i (40 mg/dl) and MT_2 (80 mg/dl). M is positive because the glycemic transition was an increase in blood sugar levels from t_1 to t_2 .

[0056] Also, for example, if MT_{min} is 40 mg/dL and if a glycemic transition is one in which the blood glucose level is 120 mg/dl at t_1 and the blood glucose level at t_2 is 20 mg/dl, then the M for this glycemic transition is -2, since 120 mg/dl subtracted from 20 mg/dl is -100 mg/dl and the number of MT the glycemic transition meets is -2; The glycemic transition meets MT_1 and MT_2 , but did not meet MT_3 (-120 mg/dL). M is a negative number because the glycemic transition was a decrease in blood glucose levels from t_1 to t_2 .

[0057] In exemplary aspects, for a given determination of an index of glucose volatility, there may be more than one MT_{min} . In exemplary aspects, when the glycemic transition starts and ends within a first range of blood glucose levels, there is a first MT_{min} , and when the glycemic transition starts and ends within a second range, there is a second MT_{min} . For example, an index of glucose volatility may be determined, wherein, when a glycemic transition starts and ends within a range of between 70 and 120 mg/dL, then a first MT_{min} (e.g., 40 mg/dL) is used and, wherein, when the glycemic transition starts and ends in range that between 1 and 70 mg/dL or between 121 and 300 mg/dL, a second MT_{min} is utilized (e.g., 10 mg/dL).

[0058] In exemplary aspects, the data evaluated are normalized or smoothed data of raw data collected and recorded from a continuous glucose monitor (CGM). In exemplary aspects, the normalized or smoothed data removes any and all glycemic transition that has an M of 0. Such glycemic transitions do not meet any magnitude thresholds. In some cases, but not all, such glycemic transitions have an M which is less than MT_{min} .

[0059] *Frequency and Total time period*

[0060] With regard to the methods described herein, the method comprises counting the number of occurrences (e.g., the frequency, F) of glycemic transitions of the same magnitude, M , within a total time period, T . In other words, the methods comprise determining the frequency (F) at which a glycemic transition of magnitude, M , occurs within a total time period, T . In exemplary aspects, T is at least or about at least or about 1 hour, at least or about 2 hours, at least or about 3 hours, at least or about 4 hours, at least or about 5 hours, at least or about 6 hours, at least or about 7 hours, at least or about 8 hours, at least or about 9 hours, at least or about 10 hours, at least or about 11 hours, at least or about 12 hours, at least or about 14 hours, at least or

about 16 hours, at least or about 18 hours, at least or about 20 hours, at least or about 22 hours, at least or about 24 hours, at least or about 48 hours, at least or about 72 hours, at least or about 4 days, at least or about 5 days, at least or about 6 days, at least or about 7 days, at least or about 2 weeks, at least or about 3 weeks, at least or about 4 weeks, at least or about 1 month, at least or about 2 months, at least or about 3 months, at least or about 4 months, at least or about 5 months, at least or about 6 months, at least or about 7 months, at least or about 8 months, at least or about 9 months, at least or about 10 months, at least or about 11 months, at least or about 12 months, or more.

[0061] For every unique M , there will be a frequency associated with that M and it may be denoted as F_M . For example, if 6 glycemic transitions occurred during time period (T) each glycemic transition of which had a magnitude (M) of -3, then F_{-3} is 6. If during that same time period (t), 3 glycemic transitions occurred each of which had an M of 4, then F_4 is 3.

[0062] W_M

[0063] The number of occurrences (e.g., the frequency, F) of glycemic transitions of the same magnitude within T is multiplied by the absolute value of the magnitude, M , to get a weighted number W_M . For example, in a total time period T which is 24 hours, if a subject had 4 occurrences of a glycemic decrease of magnitude 3, then W_{-3} is 12. If the subject also exhibited 8 occurrences of a glycemic increase of magnitude 2, then W_2 here is 16.

[0064] The index of glucose volatility is determined by summing the W_M values within a given time period. If the 4 occurrences of glycemic decrease of magnitude 3 occurred within the same 24 hour period of the 8 occurrences of a glycemic increase of magnitude 2, and these were the only glycemic transitions that occurred within the 24 hour period, then the index of glucose volatility is the sum of the two W_M values $W_{-3} + W_2 = 12 + 16$, which is 28.

[0065] In exemplary aspects, the index of glucose volatility accounts for the glycemic transitions, wherein the absolute value of M is greater than 1. Thus, for example, if data demonstrated within a 24 hour period, 4 occurrences of glycemic decrease of magnitude 3, 8 occurrences of a glycemic increase of magnitude 2, and 60 occurrences of a glycemic decrease of magnitude 1, the index of glucose volatility would only account for the two W_M values associated with the glycemic transitions having magnitude 3 and 2 (W_{M3} and W_{M2}). The 60 occurrences of a glycemic decrease of magnitude 1 would not be accounted for in the index.

[0066] *Duration, D*

[0067] For each glycemic transition which is equivalent to or greater than $MT_{m,i}$, a duration, D is determined. For a glycemic transition occurring between t1 and t2, D is determined by taking the absolute value of the difference between t1 from t2. For example, if t1 is 1 minute and t2 is 16 minutes, then the duration, D, of the glycemic transition is 15 minutes.

[0068] In exemplary embodiments, the index of glucose volatility determined in the methods described herein accounts for the occurrence of only glycemic transitions that occur within a given or predetermined or preselected range of durations. In exemplary aspects, the index of glucose volatility is the sum of W_M values associated with only those glycemic transitions having a duration of 1 hour or less. In exemplary aspects, the index of glucose volatility is the sum of W_M values associated with only those glycemic transitions having a duration between 1 and 2 hours, or 2 and 3 hours, or 3 and 4 hours.

[0069] In exemplary aspects, the index of glucose volatility is determined for a total time period (T) at least or about 24 hours, e.g., 1 or more days, 2, days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, or more, the index of glucose volatility accounts for the glycemic transitions, wherein the absolute value of M is greater than 1, and the index of glucose volatility determined in the methods described herein accounts for the occurrence of only glycemic transitions that occur within a duration (D) of 1 hour. That is to say that the absolute value of (t1 - t2) must be 60 minutes or less. In exemplary aspects, the index of glucose volatility is expressed as a daily average such that the sum of W_M values is divided by 24.

[0070] In exemplary aspects, the method of determining an index of glucose volatility comprises the steps of: (a) analyzing blood glucose level data of the subject collected and recorded at short, regular intervals, wherein the data are analyzed for an occurrence of a glycemic transition which is at least or about a minimum magnitude threshold, $MT_{m,i}$, (b) determining a magnitude, M, of each glycemic transition which is equivalent to or greater than $MT_{m,i}$, wherein M is a positive integer, when the glycemic transition is an increase, and M is a negative integer, when the glycemic transition is a decrease, wherein M is determined by counting the number of magnitude thresholds (MT) a glycemic transition meets, wherein each MT, MT_n , is fixed at a value that equals n multiplied by $MT_{m,i}$; (c) for each unique M having an absolute value greater than 1, calculating a value W_M by multiplying the value M by the number

of occurrences of glycemic transitions within a total time period, T , having that M , (d) summing each W_M for each M to obtain an index of glucose volatility.

[0071] In exemplary aspects, the index of glucose volatility accounts for only those glycemic transitions that meet a certain duration minimum. In some aspects, the duration minimum is 5 minutes. In exemplary aspects, the index of glucose volatility accounts for only those glycemic transitions that occur within a range of durations, e.g., 5 min to about 60 min, 10 min to about 30 min, 15 min to about 60 min. In exemplary aspects, the index of glucose volatility accounts for only those glycemic transitions that occur within about 1 hour. In exemplary aspects, the index of glucose volatility determined in the methods described herein accounts for the occurrence of all glycemic transitions equivalent to or greater than MT_{min} , regardless of the duration of the glycemic transition.

[0072] *Steps to Determining an Index of Glucose Volatility*

[0073] In some aspects, determining the index of blood glucose volatility comprises the steps of (a) analyzing blood glucose level data of the subject collected and recorded at short, regular intervals, wherein the data are analyzed for an occurrence of a glycemic transition which is at least or about a minimum magnitude threshold, MT_{min} , (b) determining a magnitude, M , of each glycemic transition which is equivalent to or greater than MT_{min} , wherein M is a positive integer, when the glycemic transition is an increase, and M is a negative integer, when the glycemic transition is a decrease, wherein M is determined by counting the number of magnitude thresholds (MT) a glycemic transition meets, wherein each MT , MT_n , is fixed at a value that equals n multiplied by MT_{min} ; (c) for each unique M having an absolute value greater than 1, calculating a value W_M by multiplying the value M by the number of occurrences of glycemic transitions within a total time period, T , having that M , and (d) summing each W_M for each M to obtain an index of glucose volatility.

[0074] In exemplary aspects, the index of blood glucose volatility is determined by (a) analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a

minimum magnitude threshold, MT_{min} , an F_M is determined, (b) determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1.

[0075] In some aspects, determining the index of blood glucose volatility comprises the steps of: (a) analyzing blood glucose level data of the subject for an occurrence of a glycemic transition which is at least or about a minimum magnitude threshold, MT_{min} , wherein MT_{min} is a number between 10 and 60; (b) determining the magnitude, M , of each glycemic transition which is equivalent to or greater than MT_{min} , wherein M is a positive integer, when the glycemic transition is an increase, and M is a negative integer, when the glycemic transition is a decrease, wherein M is determined by counting the number of magnitude thresholds (MT) a glycemic transition meets, wherein each MT , MT_n , is fixed at a value that equals n multiplied by MT_{min} ; (c) for each occurrence of a glycemic transition having a determined M , assigning based on the M of the glycemic transition the occurrence to one of a series of magnitude bins, wherein each magnitude bin of the series has a unique M , (d) determining the number of occurrences within each magnitude bin; (e) determining the duration, D , of each glycemic transition having a determined M , (f) for each occurrence of a glycemic transition having a determined D , assigning based on the D of the glycemic transition the occurrence to one of a series of duration bins, wherein each duration bin of the series encompasses a unique range of D values, wherein an occurrence is assigned to the duration bin having the range of D values within which the D of the occurrence falls, (g) for each magnitude bin, multiplying the magnitude, M , of the bin by the number of occurrences of glycemic transitions in that magnitude bin, thereby obtaining a number, W_M , (h) summing each W_M to obtain an index of glucose volatility, wherein the blood glucose level data was collected and recorded at short, regular intervals.

[0076] In exemplary aspects, the index of blood glucose volatility is dynamic stress factor (DySF). DySF is the daily weighted number of large monotonic glycemic transitions that occur within one hour (Rawlings et al, *Diabetes Technol Ther* 13: 1241-1248 (2011)). As such, DySF reflects both the magnitude and frequency of glucose dynamics and provides a measure of glucose volatility—that is, the likelihood of undergoing a large change in glucose level in a short amount of time. DySF can be computed using the CGM-GUIDE transition density profile, wherein the bin sizes for the separate glycemic threshold levels are set at 40 mg/dL. Note that

we are currently calculating one DySF over a span of multiple days (i.e. the entire duration of the glucose collection time period). However, we could also calculate individual DySF values for each 24-period. We could then compare DySF values across days to succinctly analyze a person's control of glucose volatility over time.

[0077] *Severe Hypoglycemic Episode*

[0078] In exemplary aspects, the methods of the invention relate to severe hypoglycemic episodes. As used herein, the term "severe hypoglycemic episode" is synonymous with "severe hypoglycemic event" and refers an occurrence wherein a blood glucose level of a subject is at or below 70 mg/dL. In exemplary embodiments, the severe hypoglycemic episode is an occurrence wherein a blood glucose level of a subject is at or below 70 mg/dL for at least or about 30 seconds (e.g., at least or about 35 seconds, at least or about 45 seconds, at least or about 60 seconds). In exemplary embodiments, the blood glucose level of a subject is at or below 70 mg/dL for at least 1 minute, at least 2 minutes, at least 3 minutes, at least 4 minutes, at least 5 minutes, at least 6 minutes, at least 7 minutes, at least 8 minutes, at least 9 minutes, or at least 10 minutes. In exemplary embodiments, the severe hypoglycemic episode is an occurrence wherein a blood glucose level of a subject is at or below 60 mg/dL, at or below 50 mg/dL, at or below 40 mg/dL, at or below 30 mg/dL, at or below 20 mg/dL, or at or below 10 mg/dL. In exemplary aspects, the severe hypoglycemic episode is an occurrence of blood glucose level at or below 70 mg/dL and one or more of the following physiological manifestations of this blood glucose level: fainting, light headedness, dizziness, headache, seizure, coma, shock.

[0079] The invention provides a method of determining a subject's risk for a severe hypoglycemic episode. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to be at risk for a severe hypoglycemic episode.

[0080] The invention also provides a method of determining a subject's need for prophylaxis of a severe hypoglycemic episode. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to need prophylaxis of a severe hypoglycemic episode. As used herein, a "prophylaxis of a severe

hypoglycemic episode" is any compound that prevents a hypoglycemic episode and includes for example, glucagon, or any compound that reduces blood glucose volatility.

[0081] The invention further provides a method of preventing a severe hypoglycemic episode in a subject. In exemplary embodiments, the method comprises the steps of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject prophylaxis for a severe hypoglycemic episode in an amount sufficient to prevent the severe hypoglycemic episode, when the index of blood glucose volatility is decreased, as compared to a control index.

[0082] The invention also provides a method of reducing a subject's risk for a severe hypoglycemic episode in a subject. In exemplary embodiments, the method comprises the step of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject a therapeutic agent for the treatment of diabetes, or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0083] The invention provides a method of monitoring a subject's risk for a severe hypoglycemic episode. In exemplary embodiments, the method comprises the steps of determining a first index of blood glucose volatility of the subject at a first time point and determining a second index of blood glucose volatility of the subject at a second time point, wherein the first time point occurs before the second time point, wherein the subject's risk for a severe hypoglycemic episode is increased, when the second index is less than the first index.

[0084] In the above methods relating to severe hypoglycemic episodes, the index of blood glucose volatility is determined through any of the methods of determining an index of blood glucose volatility described herein.

[0085] *Long term complications of Diabetes*

[0086] In exemplary aspects, the methods of the invention relate to long term complication of diabetes. As used herein, the term "long term complication of diabetes" refers to a chronic medical condition, disease, or disorder caused by long-term affliction with diabetes. In exemplary aspects, the long term complication of diabetes is a vascular disease, e.g., a microvascular disease, a macrovascular disease. In exemplary aspects, the long term complication of diabetes is one of the following: (i) Diabetic vascular disease (e.g., the development of blockages in the arteries, which can lead to foot ulcers, infections, and even loss

of a toe, foot, or lower leg, heart attack, stroke, blockage of blood vessels in legs and feet); (ii) Diabetic Retinopathy (e.g., abnormal growth of blood vessels in your retina); (iii) glaucoma; (iv) cataracts; (v) Diabetic Neuropathy (e.g., nerve disorder caused by diabetes); (vi) Diabetic Nephropathy (e.g., kidney disease or damage that occurs in people with diabetes); and (vii) Periodontal disease. Some patients are more prone or at higher risk of developing a long-term complication of diabetes than others. Without being bound to any particular theory, the index of glucose volatility described herein is capable of identifying patients who are at higher risk of developing a long-term complication of diabetes over patients who are not at higher risk. Accordingly, the invention provides a method of determining a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to be at an increased risk for a long term complication of diabetes, as compared to a control subject.

[0087] The invention provides a method of determining a subject's need for prophylaxis of a long term complication of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control, the subject is determined to need prophylaxis of a long term complication of diabetes. As used herein, "prophylaxis of a long term complication of diabetes" includes any known standard of care for diabetes, many of which are described herein below. In exemplary aspects, the subject's need for a more aggressive therapeutic regimen for diabetes is determined.

[0088] The invention provides a method of determining a subject's need for increased or more frequent monitoring for a long term complication of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control, the subject is determined to need increased or more frequent monitoring for a long term complication of diabetes.

[0089] The invention additionally provides a method of preventing a long term complication of diabetes in a subject. In exemplary embodiments, the method comprises the steps of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the

subject a therapeutic agent for the treatment of diabetes or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0090] The invention also provides a method of reducing a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the step of (i) determining an index of blood glucose volatility of the subject and (ii) administering to the subject a therapeutic agent for the treatment of diabetes, or a compound effective for reducing blood glucose volatility, when the index is decreased as compared to a control index of blood glucose volatility.

[0091] The invention provides a method of monitoring a subject's risk for a long term complication of diabetes. In exemplary embodiments, the method comprises the steps of determining a first index of blood glucose volatility of the subject at a first time point and determining a second index of blood glucose volatility of the subject at a second time point, wherein the subject's risk for a long term complication of diabetes is increased, when the second index is less than the first index. In the above methods relating to long term complications of diabetes, the index of blood glucose volatility is determined through any of the methods of determining an index of blood glucose volatility described herein.

[0092] *Additional Methods*

[0093] The invention further provides a method of determining the efficacy of a compound for reducing blood glucose volatility in a subject. In exemplary embodiments, the method comprises the steps of (i) determining a first index of blood glucose volatility of the subject before administration of the compound and (ii) determining a second index of blood glucose volatility of the subject after administration of the compound, wherein the compound is determined as effective for reducing blood glucose volatility, when the second index is higher than the first index.

[0094] The invention furthermore provides a method of determining a therapeutic regiment for a subject (e.g., a diabetic subject). The method comprises the step of determining an index of blood glucose volatility of the subject. When the index of blood glucose volatility is decreased, as compared to a control index, the subject is determined to need a more aggressive therapeutic regiment.

[0095] The invention further provides a method of treating diabetes in a subject. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject and administering to the subject a therapeutic agent for the treatment of diabetes, when the index of blood glucose volatility is decreased, as compared to a control index.

[0096] The invention also provides a method of determining a subject's need for a therapeutic agent for the treatment of diabetes. In exemplary embodiments, the method comprises the step of determining an index of blood glucose volatility of the subject, wherein the subject is in need for a therapeutic agent for the treatment of diabetes or a compound that reduces blood glucose volatility, when the index of blood glucose volatility is decreased, as compared to a control index.

[0097] The invention provides a method of monitoring the advancement of diabetes in a subject. In exemplary embodiments, the method comprises the steps of determining a first index of blood glucose volatility of the subject at a first time point and determining a second index of blood glucose volatility of the subject at a second time point, wherein the first time point occurs before the second time point, wherein the diabetes has advanced, when the second index is less than the first index.

[0098] In the above methods relating to diabetes, the index of blood glucose volatility is determined through any of the methods of determining an index of blood glucose volatility described herein.

[0099] *Treatment and Prevention*

[00100] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment or prevention in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the disease being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[00101] *Subjects*

[00102] With regard to the methods of the invention, the subject may be any multicellular, eukaryotic organism of the kingdom Animalia or Metazoa. In exemplary aspects, the subject is of the Chordata phylum. In some embodiments, the subject is a mammal. As used herein, the term "mammal" refers to any vertebrate animal of the mammalia class, including, but not limited to, any of the monotreme, marsupial, and placental taxas. In some embodiments, the mammal is one of the mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. In exemplary embodiments, the mammals are from the order Carnivora, including Felines (cats) and Canines (dogs). In exemplary embodiments, the mammals are from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perissodactyla, including Equines (horses). In some instances, the mammals are of the order Primates, Ceboidea, or Simiiformes (monkeys) or of the order Anthropoidea (humans and apes). In particular embodiments, the mammal is a human.

[00103] In exemplary aspects, the subject is a human adult subject, e.g., a subject aged more than about 18 years of age. In exemplary aspects, the subject is a human child, e.g., a subject aged less than 18 years. In exemplary aspects, the subject is less than 17 years old, less than 16 years old, less than 15 years old, or less than about 14 years. In exemplary aspects, the subject is aged between about 5 and about 18 years, or about 5 and 17 years, or about 7 and about 16 years, or about 5 and 15 years, or about 5 and 14 years, or about 5 and 13 years, or about 5 and 12 years, or about 5 and 11 years, or about 5 and 10 years, or about 6 and 18 years, or about 6 and 17 years, or about 6 and 16 years, or about 6 and 15 years, or about 6 and 14 years, or about 6 and 13 years, or about 6 and 12 years, or about 6 and 11 years, or about 6 and 10 years, or about 7 and 18 years, or about 7 and 17 years, or about 7 and 16 years, or about 7 and 15 years, or about 7 and 14 years, or about 7 and 13 years, or about 7 and 12 years, or about 7 and 11 years, or about 7 and 10 years, or about 8 and 18 years, or about 8 and 17 years, or about 8 and 16 years, or about 8 and 15 years, or about 8 and 14 years, or about 8 and 13 years, or about 8 and 12 years, or about 8 and 11 years, or about 8 and 10 years, or about 9 and 18 years, or about 9 and 17 years, or about 9 and 16 years, or about 9 and 15 years, or about 9 and 14 years, or about 9 and 13 years, or about 9 and 12 years, or about 9 and 11 years, or about 9 and 10 years, or about 10 and 18 years, or about 10 and 17 years, or about 10 and 16 years, or about 10 and 15 years.

[00104] In exemplary aspects, the subject is a suffers from a metabolic disease. In exemplary aspects, the metabolic disease is metabolic syndrome. Metabolic Syndrome, also known as metabolic syndrome X, insulin resistance syndrome or Reaven's syndrome, is a disorder that affects over 50 million Americans. Metabolic Syndrome is typically characterized by a clustering of at least three or more of the following risk factors: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders including high triglycerides, low HDL cholesterol and high LDL cholesterol that enhance the accumulation of plaque in the artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance, (5) prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor- 1 in blood), and (6) pro-inflammatory state (e.g., elevated C-reactive protein in blood). Other risk factors may include aging, hormonal imbalance and genetic predisposition.

[00105] Metabolic Syndrome is associated with an increased the risk of coronary heart disease and other disorders related to the accumulation of vascular plaque, such as stroke and peripheral vascular disease, referred to as atherosclerotic cardiovascular disease (ASCVD). Patients with Metabolic Syndrome may progress from an insulin resistant state in its early stages to full blown type II diabetes with further increasing risk of ASCVD. Without intending to be bound by any particular theory, the relationship between insulin resistance, Metabolic Syndrome and vascular disease may involve one or more concurrent pathogenic mechanisms including impaired insulin-stimulated vasodilation, insulin resistance-associated reduction in NO availability due to enhanced oxidative stress, and abnormalities in adipocyte-derived hormones such as adiponectin (Lteif and Mather, Can. J. Cardiol. 20 (suppl. B):66B-76B (2004)).

[00106] According to the 2001 National Cholesterol Education Program Adult Treatment Panel (ATP III), any three of the following traits in the same individual meet the criteria for Metabolic Syndrome: (a) abdominal obesity (a waist circumference over 102 cm in men and over 88 cm in women); (b) serum triglycerides (150 mg/dl or above); (c) HDL cholesterol (40 mg/dl or lower in men and 50 mg/dl or lower in women); (d) blood pressure (130/85 or more); and (e) fasting blood glucose (110 mg/dl or above). According to the World Health Organization (WHO), an individual having high insulin levels (an elevated fasting blood glucose or an elevated post meal glucose alone) with at least two of the following criteria meets the criteria for Metabolic Syndrome: (a) abdominal obesity (waist to hip ratio of greater than 0.9, a body mass index of at least 30 kg/m², or a waist measurement over 37 inches); (b) cholesterol panel

showing a triglyceride level of at least 150 mg/dl or an HDL cholesterol lower than 35 mg/dl; (c) blood pressure of 140/90 or more, or on treatment for high blood pressure). (Mathur, Ruchi, "Metabolic Syndrome," ed. Shiel, Jr., William C, MedicineNet.com, May 11, 2009).

[00107] For purposes herein, if an individual meets the criteria of either or both of the criteria set forth by the 2001 National Cholesterol Education Program Adult Treatment Panel or the WHO, that individual is considered as afflicted with Metabolic Syndrome.

[00108] In exemplary aspects, the metabolic disease is a hyperglycemic medical condition. In exemplary aspects, the hyperglycemic medical condition is diabetes, diabetes mellitus type I, diabetes mellitus type II, or gestational diabetes, either insulin-dependent or non-insulin-dependent. In some aspects, the method treats the hyperglycemic medical condition by reducing one or more complications of diabetes including nephropathy, retinopathy and vascular disease.

[00109] In exemplary aspects, the subject exhibits (i) a mean HbA1c level below or about 6.5 (e.g., below or about 6.4, below or about 6.3, below or about 6.2, below or about 6.1, below or about 6.0, below or about 5.9, below or about 5.8, or below or about 5.7), or (ii) a mean glucose level of about 140 mg/dL or below (e.g., about 130 mg/dL or below, about 120 mg/dL or below, about 110 mg/dL or below, about 100 mg/dL or below, about 90 mg/dL or below, about 80 mg/dL or below, or about 70 mg/dL or below). In exemplary aspects, the subject exhibits a mean HbA1C level below 6. In exemplary aspects, the subject exhibits a mean glucose level between about 70 and about 80 mg/dL.

[00110] *Control Indices*

[00111] In regards to the methods of the invention, the index of blood glucose volatility is compared to a control index of a control subject. In some embodiments, the control subject is a matched control of the same species, gender, ethnicity, age group, smoking status, BMI, current therapeutic regimen status, medical history, or a combination thereof, but differs from the subject for whom the risk is being determined or monitored or reduced or the need for intervention is being determined in that the control does not suffer from a severe hypoglycemic episode, a long term complications of diabetes, or diabetes, or hypoglycemia, and/or does not have a history inclusive of a severe hypoglycemic episode, a long term complications of diabetes or diabetes, or hypoglycemia. In exemplary aspects, the control index of blood glucose volatility is an index of blood glucose volatility of a population of subjects known to not suffer from a severe hypoglycemic episode or a long term complication of diabetes. In exemplary aspects, the control

index is an index of a population of subjects known to not suffer from diabetes or other metabolic disease. In exemplary aspects, the control index of blood glucose volatility is an index of blood glucose volatility of the same individual but taken at an earlier time point earlier.

[00112] Relative to a control index, the index of blood glucose volatility that is determined may be an increased level. As used herein, the term "increased" with respect to an index of blood glucose volatility refers to any % increase above a control index. The increase may be at least or about a 5% increase, at least or about a 10% increase, at least or about a 15% increase, at least or about a 20% increase, at least or about a 25% increase, at least or about a 30% increase, at least or about a 35% increase, at least or about a 40% increase, at least or about a 45% increase, at least or about a 50% increase, at least or about a 55% increase, at least or about a 60% increase, at least or about a 65% increase, at least or about a 70% increase, at least or about a 75% increase, at least or about a 80% increase, at least or about a 85% increase, at least or about a 90% increase, at least or about a 95% increase, relative to a control index. In exemplary aspects, the increase is at least a 50% increase over the control index. The increase may be a 1.1-fold, a 1.2-fold, a 1.3-fold, a 1.4-fold, a 1.5-fold, a 1.6-fold, a 1.7-fold, a 2.0-fold, a 2.5-fold, a 3.0 fold, a 3.5-fold, a 4.0-fold, a 4.5-fold, a 5.0 fold, a 6.0 fold, a 7.0-fold, an 8.0-fold, a 9.0-fold, 10-fold, a 15-fold, a 20-fold, a 30-fold, a 40-fold, a 50-fold or more, increase, relative to the control index.

[00113] Relative to a control index, the index of blood glucose volatility that is determined may be a decreased level. As used herein, the term "decreased" with respect to an index of blood glucose volatility refers to any % decrease below a control index. The decrease may be at least or about a 5% decrease, at least or about a 10% decrease, at least or about a 15% decrease, at least or about a 20% decrease, at least or about a 25% decrease, at least or about a 30% decrease, at least or about a 35% decrease, at least or about a 40% decrease, at least or about a 45% decrease, at least or about a 50% decrease, at least or about a 55% decrease, at least or about a 60% decrease, at least or about a 65% decrease, at least or about a 70% decrease, at least or about a 75% decrease, at least or about a 80% decrease, at least or about a 85% decrease, at least or about a 90% decrease, at least or about a 95% decrease, relative to a control index. In exemplary aspects, the decrease is at least a 50% decrease below the control index. The decrease may be a 1.1-fold, a 1.2-fold, a 1.3-fold, a 1.4-fold, a 1.5-fold, a 1.6-fold, a 1.7-fold, a 2.0-fold, a 2.5-fold, a 3.0 fold, a 3.5-fold, a 4.0-fold, a 4.5-fold, a 5.0 fold, a 6.0 fold, a 7.0-fold, an 8.0-fold,

a 9.0-fold, 10-fold, a 15-fold, a 20-fold, a 30-fold, a 40-fold, a 50-fold or more, decrease, relative to the control index.

[00114] *Additional steps*

[00115] In some embodiments of the methods provided herein, the method comprises additional steps or comprises a combination of the steps disclosed herein. In some embodiments, steps of the inventive method are repeated one or more times. In exemplary aspects, the method comprises determining the index of blood glucose volatility of the subject at a first time point and determining the index of blood glucose volatility of the subject at a second time point, wherein the first time point occurs before the second time point. The index of blood glucose volatility of the subject may be monitored in this sense. In exemplary aspects, the method comprises repeating the step of determining the index of blood glucose volatility but with a different parameter, e.g., a different duration, total time period, minimum magnitude threshold.

[00116] In exemplary aspects, the methods are purposed for determining efficacy of a compound or screening test compounds. In exemplary aspects, the method comprises re-determining the index of blood glucose volatility of the subject after a therapeutic or prophylactic agent has been administered to the subject. In exemplary aspects, the method comprises determining the index of blood glucose volatility of the subject before and after a test agent has been administered to the subject, wherein the efficacy of the test agent as a prophylaxis of a severe hypoglycemic episode or of a long term complication of diabetes is determined.

[00117] In some embodiments, the method comprises additional steps which further characterize the subject. In exemplary aspects, the method comprises determining a subject's body mass index (BMI) , blood pressure, genetic profiles, smoking status, diet or food intake, body fat, weight, exercise practice, HbA1c levels, and the like. In exemplary aspects, the method further comprises determining a metric of blood glucose level different from the index of blood glucose volatility.

[00118] In exemplary embodiments, the methods of the invention further comprises collecting at short, regular intervals blood samples of the subject to determine blood glucose levels at each interval over a time period. The time period may be any of those described herein. In exemplary aspects, the methods further comprise implanting a CGM in the subject and obtaining data from the CGM to obtain blood glucose level data of the subject.

[00119] In exemplary aspects, the methods of the invention comprise further steps of informing an individual (e.g., the subject from whom the biological sample was obtained, a medical practitioner, a medical insurance provider, etc.) of the determined level of blood glucose volatility. In exemplary aspects, a medical practitioner of the subject from whom the biological sample is obtained, is informed and the medical practitioner prescribes a therapeutic agent for the treatment of diabetes or a compound effective for reducing blood glucose volatility to the subject. In exemplary aspects, the subject from whom the biological sample is obtained, is informed, and the subject begins a routine of regular monitoring of risk for severe hypoglycemic episodes or long term complications of diabetes and/or begins treatment (e.g., prophylactic treatment) for severe hypoglycemic episodes or long term complications of diabetes.

[00120] In exemplary aspects, the inventive systems, computer-readable storage media, methods implemented by a processor in a computer, or a combination thereof, each of which described below, may be utilized in any of the methods of the invention.

[00121] In exemplary aspects of the inventive methods, the methods further comprise the step of administering to the subject a therapeutic agent for the treatment of diabetes, compounds that reduce blood glucose volatility, or prophylaxis of a severe episode of hypoglycemia or prophylaxis of a long term complication of diabetes, when the index of blood glucose volatility is decreased, relative to a control index. Suitable therapeutic agents for the treatment of diabetes, compounds that reduce blood glucose volatility, or prophylaxes of a severe episode of hypoglycemia or prophylaxes of a long term complication of diabetes are known in the art, some of which are described below.

[00122] *Additional metabolic metrics*

[00123] In exemplary aspects, the method further comprises determining one or more of the following of the subject: a mean of daily differences (MODD), a continuous overall net glycemic action (CONGA(n)), a mean amplitude of glycemic excursion (MAGE), an M-value, an average daily risk range (ADRR), a level of Hb1Ac, a point-error grid analysis (P-EGA), a Rate-error grid analysis (R-EGA), or a continuous glucose error grid analysis (CG-EGA).

[00124] **Mean of daily differences (MODD)** is the mean of all valid absolute value differences between glucose concentrations measured at the same time of day on two consecutive days (Molnar et al, *Diabetologia* 8: 342-348 (1972)). MODD therefore reflects

inter-day glucose variation and can be considered an index for daily glucose consistency (McDonnell et al, *Diabetes Technol Ther* 7(2): 253-263 (2005)). Unlike DySF, MODD does not consider clinically-defined ranges of glycemic states (hyperglycemic, hypoglycemic, and euglycemic).

[00125] Continuous overall net glycemic action (CONGA(n)) is the standard deviation of all valid differences between a current observation and an observation (n) hours earlier (McDonnell et al, *Diabetes Technol Ther* 7: 253-263 (2005)). The value of n is typically taken to be 1, 4, or 8. By specifying $n < 24$, CONGA measures intra-day glucose variation. CONGA has been described as a measure of glucose lability (McDonnell et al, *Diabetes Technol Ther* 7(2): 253-263(2005)), or the likelihood of undergoing a change in glucose level over a defined length of time. While CONGA reflects the variability of differences in glucose levels, it does not specifically address large glucose transitions, as does DySF. For example, mathematically, it is possible to have a large CONGA value for glucose levels that remain in euglycemic ranges. This may lead to a false deduction of the presence of numerous glucose excursions into hyper- or hypo- glycemic states. Using DySF as an index of glycemic control would not lead to this kind of erroneous inference.

[00126] Mean amplitude of glycemic excursion (MAGE) is the arithmetic average of absolute value differences between adjacent glucose peaks and nadirs, where the differences exceed 1 SD from the mean (Service et al, *Diabetes* 19:644-655 (1970)). (Algorithm described in Fritzsche et al, *Diabetes Technol Ther* 13(3): 319-325 (2011)). MAGE is similar to DySF in the sense that it is a measure of fluctuation severity. However, unlike DySF, MAGE does not take into account the time interval over which these glucose fluctuations occur.

[00127] M-value was one of the earliest glucose lability metrics to be developed. The formula for M-value was empirically determined, and its calculation requires the specification of an arbitrary ideal glucose value (IGV). The authors who developed M-value described it as a quantitative index of the lack of efficacy of insulin or other therapeutic treatment in the diabetic individual (Schlichtkrull et al, *Acta Med Scand* 177:95-102 (1965)). The metric was made to be used with self-monitored finger-prick glucose data, not continuous glucose data.

[00128] Average daily risk range (ADRR) is calculated as the average of daily [blood glucose range] over 30 days, but the glucose data are first normalized and converted into their

corresponding risk values (Kovatchev et al, *Diabetes Care* 29: 2433-2438 (2006)). Like M-value, the normalizing function used to calculate ADRR was empirically determined, and the metric is designed for analyzing self-monitored blood glucose (SMBG) data. In fact, between 14 and 30 days' worth of data with at least 3 SMBG readings per day must be analyzed at a time.

[00129] HbA1c reflects the average blood glucose level over the past six to eight weeks. In healthy people, the HbA1c level is less than 6% of total hemoglobin. Studies have demonstrated that the complications of diabetes can be delayed or prevented if the HbA1c level can be kept below 7%. A severe limitation of HbA1c is that since it is not influenced by daily fluctuations in blood glucose, it cannot be used to monitor day-to-day blood glucose levels and to adjust insulin doses, nor can it detect the day-to-day presence or absence of hyperglycemia or hypoglycemia. Research has shown, however, that glucose variability on the time-scale of days as opposed to months provides critical information one needs to better analyze and treat diabetic conditions. We showed that DySF distinguishes glucose variability populations within strata (either ≥ 7 or < 7) of HbA1c. DySF is therefore a more precise indicator of glucose dynamics than HbA1c.

[00130] (Hemoglobin is the predominant protein in red blood cells and is the oxygen-carrying pigment that gives blood its red color. About 90% of hemoglobin is of type "A". Hemoglobin A_{1c} is a minor component of hemoglobin A to which glucose is bound. The hemoglobin A_{1c} blood test (HbA1c) measures the concentration of hemoglobin A_{1c}, and therefore reflects the amount of glucose in the blood. Levels of HbA1c are not influenced by daily fluctuations in the blood glucose concentration but reflect the average glucose levels over the prior six to eight weeks.)

[00131] Point-error grid analysis (P-EGA) is a plot of continuous glucose monitor (CGM) readings versus reference glucose readings (Kovatchev et al, *Diabetes Care* 27: 1922-1928 (2004)). The plot is divided into several zones. Each zone corresponds to a certain degree of "danger" based on considerations of what clinical outcome might occur if the patient took action based on CGM feedback about glucose levels.

[00132] Rate-error grid analysis (R-EGA) is analogous to P-EGA, but used to assess glucose rate of change rather than glucose point readings.¹⁰ R-EGA is thus a plot of the rates of change of CGM-data versus the rates of change of reference glucose readings. The plot is divided into several zones. Each zone corresponds to a certain degree of "danger" based on

considerations of what clinical outcome might occur if the patient took action based on CGM feedback about the direction and rate of glucose fluctuations.

[00133] Continuous glucose error grid analysis (CG-EGA) combines P-EGA and R-EGA to appraise the ability of CGMs to accurately report blood glucose readings and the direction and rate of change of glucose levels (Kovatchev et al, *Diabetes Care* 27: 1922-1928 (2004)). CG-EGA focuses on the clinical implications of measurement error and evaluates the accuracy of CGMs to prompt appropriate clinical action by the patient. CG-EGA is not intended to study the accuracy of long-term trends depicted by CGMs. CG-EGA is distinct from DySF in that CG-EGA measures the accuracy of the CGM data, whereas DySF takes presumably accurate CGM data and uses that data to assess glucose fluctuation dynamics.

[00134] Therapeutic agents

[00135] Therapeutic agents for the treatment of diabetes are known in the art and include but not limited to insulin, leptin, Peptide YY (PYY), Pancreatic Peptide (PP), fibroblast growth factor 21 (FGF21), Y2Y4 receptor agonists, sulfonylureas, such as tolbutamide (Orinase), acetohexamide (Dymelor), tolazamide (Tolinase), chlorpropamide (Diabinese), glipizide (Glucotrol), glyburide (Diabeta, Micronase, Glynase), glimepiride (Amaryl), or gliclazide (Diamicon); meglitinides, such as repaglinide (Prandin) or nateglinide (Starlix); biguanides such as metformin (Glucophage) or phenformin; thiazolidinediones such as rosiglitazone (Avandia), pioglitazone (Actos), or troglitazone (Rezulin), or other PPAR γ inhibitors; alpha glucosidase inhibitors that inhibit carbohydrate digestion, such as meglitol (Glyset), acarbose (Precose/Glucobay); exenatide (Byetta) or pramlintide; Dipeptidyl peptidase-4 (DPP-4) inhibitors such as saxagliptin, vildagliptin or sitagliptin; SGLT (sodium-dependent glucose transporter 1) inhibitors; glucokinase activators (GKA); glucagon receptor antagonists (GRA); or FBPase (fructose 1,6-bisphosphatase) inhibitors.

[00136] Compounds effective for reducing blood glucose volatility include but are not limited to insulin and somatostatins. It has been suggested that PGX® or PolyGlycopleX® (a novel complex of water soluble polysaccharides (plant fibers)) lowers glucose volatility.

[00137] *Systems, computer-readable storage medium, and methods implemented by a processor in a computer*

[00138] Figure 9 depicts a system 100 for calculating and/or monitoring DySF. The system 100 includes a device 102 for collecting and/or storing CGM data and for performing calculations to determine DySF based on the collected and/or stored data. The device 102 receives CGM data from one or more CGM data sources 104-110. The device 102 may be any of a variety of devices, including a dedicated purpose glucose monitor, a smart phone, a laptop computer, a tablet, computer, a wearable monitor, an insulin pump, a hospital-based monitoring system or device, etc.

[00139] The CGM data sources 104-110 can be any of a variety of sources and, in particular, any source that collects or stores CGM data to be provided to the device 102. For example, a sub-dermal or subcutaneous glucose sensor 104. In some embodiments, the sensor 104 transmits glucose measurements wirelessly to the device 102, while in other embodiments, the sensor 104 is wired to the device 102. Regardless of whether CGM data are transmitted to the device 102 wireless or via a wired connection, the sensor 104 may, in various embodiments, communicate the CGM data to the device 102 continuously (i.e., as the data are collected), in bursts (e.g., every half hour), as a cumulative set of data (e.g., at the end of a 24-hour monitoring cycle), or upon removal of the sensor 104. In another embodiment, a smart patch 106 collects blood glucose data transdermally and stores and/or transmits the collected data to the device 102. Like the sensor 104, the smart patch 106 may communicate data to the device 102 wirelessly or via a wired connection, and may communicate data continuously, in bursts, etc.

[00140] In embodiments in which the sensor 104 or the smart patch 106 communicate CGM data to the device 102 wirelessly, the wireless communication may be via any known or future suitable transmission medium and/or protocol. By way of example and not limitation, the device 102 may receive CGM data using one of the IEEE 802.11 protocols (i.e., a "WiFi" protocol), Bluetooth™, Near Field Communication ("NFC"), etc. Additionally, in some embodiments, especially where the device 102 is a smart phone, the device 102 may receive CGM data via an internet connection, via Short Message Service (SMS), or via a mobile data signal (e.g., LTE, WiMax, etc.). In certain embodiments in which the sensor 104 or the smart patch 106 communicate CGM data to the device 102 via a wired connection, the connection may be any appropriate data connection including, for example, connections implementing the IEEE 1394 protocol, a Universal Serial Bus (USB) protocol, etc.

[00141] Additionally, the smart patch 106 may include a removable storage device such as a memory card 108 or a USB storage device 110 that may store CGM data collected by the smart patch 106. The device 102 may include a storage device reader or an interface for coupling to the memory card 108 or the USB storage device 110. Of course, in embodiments in which data are transferred to the device 102 by a memory card 108 or by a USB storage device 110, the data are transferred in a single data dump, rather than continuously or in bursts.

[00142] While the device 102 may be any of a variety of devices, as described above, the device 102 may also communicate with any of a variety of other devices 112-118. That is, the device 102 may communicate DySF data and/or CGM data to the other devices 112-118 that, by way of example, may include desktop computers 112, laptop computers 114, smart phones 116, tablet computers 118, or any other computing device. The device 102 may communicate with the other devices 112-118 via a wired or wireless connection, and may transmit data over the wired or wireless connection by means of a direct connection (e.g., WiFi, Bluetooth, etc.) or using some intermediary connection such as the Internet or a mobile data carrier using e-mail, SMS, online file storage, or any other service. Of course, DySF data may also be transferred from the device 102 to one of the devices 112-118 by a memory card or a USB storage device.

[00143] Figure 10 depicts a block diagram of the device 102. The device 102 includes a processor 120. The processor 120 may be any processing device including a multi-core processing device, a single-core processing device, a digital signal processor (DSP), a general purpose processor, a specialized processor, an application specific integrated circuit (ASIC), a programmed field programmable gate array (FPGA), etc. Additionally, while depicted in Figure 10 as a single processor 120, the device 102 may implement multiple processors 120. When implemented as a general purpose processor or other multi-purpose processor (i.e., not as an ASIC or an FPGA), the processor 120 may be specially programmed to perform the operations associated with receiving CGM data and calculating and/or monitoring DySF.

[00144] The processor 120 is communicatively coupled to a memory subsystem 122. The memory subsystem 122 includes both a non-volatile memory 124 and, in some embodiments, a volatile memory 126. Of course, in many current computing devices, tasks formerly associated with volatile memory (e.g., with random access memories), such as data storage for executing computations, are implemented on non-volatile memories. In any event, the non-volatile

memory 124 may be any known non-volatile memory, including flash memory, magnetic storage devices (e.g., hard disks), optical storage (e.g., CD, DVD), FeRAM, CDRAM, PRAM, RRAM, SONOS, Racetrack memory, NRAM, etc. The non-volatile memory 122 stores one or more routines 128 (e.g., sets of computer executable instructions) for execution by the processor 120. The routines 128 may include: routines for retrieving/receiving CGM data from an external device; routines for calculating DySF; routines for calculating other metrics associated with blood glucose levels; routines for outputting data to another device, a display, or a printer; routines for programming a CGM monitoring device such as the sensor 104 or the patch 106; and/or routines for performing any other desired function(s).

[00145] In some embodiments, the non-volatile memory 122 may also include a data store 130 in which the processor 120 may store CGM data received from the devices 104-110 and/or DySF data calculated by the processor 120 and/or other data. Generally, during execution of the routines 128, the processor 120 may store data in the volatile memory 126 for fast access to the data during calculations. However, as improvements in memory technology have resulted in faster non-volatile memory devices, the processor 120 may, in some embodiments, store data for random access in the non-volatile memory 124 instead of in the volatile memory 126.

[00146] The processor 120 is also communicatively coupled to an input/output (I/O) interface 132. The I/O interface 132 includes the physical interfaces (connectors and other hardware) and logical interfaces necessary to communicate with other devices (not shown) and, in particular, may include: interfaces to input devices such as keyboards, pointing devices, touch-sensitive displays, microphones, video capture devices, etc.; interfaces to output devices such as printers, speakers, displays (internal or external), etc; communication interfaces such as WiFi, Bluetooth, Near Field Communication, USB, IEEE 1394, Ethernet, etc.; and interfaces such as readers for Secure Digital (SD) cards, Compact Flash (CF) cards, etc.

[00147] As described above, the routines 128 stored in the non-volatile memory 124 may include a routine for calculating DySF. The routine for calculating DySF may include computer-executable instructions for performing a method. Figure 11 depicts a flow chart for an exemplary method 150 for computing DySF. Each of the blocks depicted in the flow chart may represent an instruction or set of instructions. The method may be embodied in a single routine or in multiple routines. As just one example, the block 152 may be embodied as a separate

routine (as described above) for receiving CGM data, or may be part of a larger routine for calculating DySF data.

[00148] In any event, the method 150 commences when the processor 120 receives or retrieves CGM data (block 152). The processor 120 may receive/retrieve the CGM data directly from a measurement device such as the sensor 104 or the smart patch 106, or may retrieve the CGM data stored in the memory subsystem 122. The CGM data include data points for a period of time between a first time and a second time. Each of the data points include at least a blood glucose measurement value and a time value. In an embodiment, the time value associated with each data point could be a time value relative to a start time of data collection (e.g., 0:35, 2:35, etc.). In an alternate embodiment, the time value associated with each data point is an absolute time (e.g., 0200, 2:00 AM, 2:00 AM December 4, etc.).

[00149] After receiving/retrieving the CGM data, the processor 120 determines the transition points in the data (block 154). The transition points are the points at which the blood glucose level changes from increasing to decreasing, or decreasing to increasing, relative to the last data point. In some embodiments, the processor 120, in determining transition points, ignores changes that do not meet certain criteria. For example, the processor 120 may ignore transition points associated with a non-monotonicity that does not cross at least one of a plurality of thresholds. With reference to Figure 12, a graph 170 depicts an example set of CGM data. Dashed lines 172 represent several threshold values. A line 174 represents a set of CGM data points, including several points 176-188 at which the line 174 changes from a positive slope to a negative slope or from a negative slope to a positive slope. Each of the points 176-188 may represent a transition point according to the method 150. However, between the point 180 and the point 182, the CGM data do not cross one of the thresholds 172. Accordingly, in some embodiments, the point 182 is not considered a transition point, and the points 180 and 184 would be considered adjacent transition points (i.e., the non-monotonicity represented by the point 182 is ignored).

[00150] The processor 120 selects a pair of successive transition points (block 156). By way of example, and referring again to Figure 12, the points 176 and 178 are successive transition points. For each pair of successive transition points, the processor 120 determines a magnitude of the difference between the measurement values associated with the pair of transition points

and whether the blood glucose level is increasing or decreasing between the pair of transition points (block 58). In an embodiment, the magnitude of the difference is an integer values selected from a group of integer values. In some embodiments, for example, the magnitude of the difference may reflect the number of thresholds crossed between the transition points. Referring again to Figure 12, the magnitude of the change between the points 176 and 178 may be two (2) in these embodiments, while the magnitude of the change between the points 180 and 184 may be one (1) in these embodiments.

[00151] In an embodiment, the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_i = (X_{2,i} / A) - (X_{1,i} / A)$$

where Y_i is the magnitude of the difference between the measurement values associated with the pair of transition points, A is a predetermined bin size, and $X_{1,i}$ and $X_{2,i}$ are a pair of successive transition points. In an embodiment, the predetermined bin size A is between 10 and 60 mg/dL and, in a particular embodiment, the bin size A is 40 mg/dL.

[00152] In another embodiment, the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_i = [(X_{2,i} / A) - \text{mod}(X_{2,i} / A)] - [(X_{1,i} / A) - \text{mod}(X_{1,i} / A)]$$

where Y_i is the magnitude of the difference between the measurement values associated with the pair of transition points, A is a predetermined bin size, and $X_{1,i}$ and $X_{2,i}$ are a pair of successive transition points. In an embodiment, the predetermined bin size A is between 10 and 60 mg/dL and, in a particular embodiment, the bin size A is 40 mg/dL.

[00153] The processor 120 also determines a time elapsed between the transition points (block 160). If there are additional pairs of successive transition points (block 62), the processor 120 repeats the blocks 156 through 160.

[00154] If there are no additional pairs of successive transition points (block 62), the processor 120 determines for each magnitude how many transitions occurred within a predetermined time. Put another way, the processor 120 determines the number of transition point pairs having an elapsed time between the transition points of less than a predetermined value and a particular magnitude. For example, the processor 120 may determine that seven transition point pairs had a

magnitude of two, but that only three of the transition point pairs with magnitude seven had an elapsed time between the pair of transition points of less than one hour. The processor 120 may make similar determinations for each magnitude. In an embodiment, the predetermined time is between 30 and 150 minutes and, in a particular embodiment, the predetermined time is 60 minutes.

[00155] The processor 120 proceeds to determine an average daily frequency of changes of each magnitude (block 66). In an embodiment, the processor 120 determines the average daily frequency F_Z for each magnitude according to the equation:

$$F_Z = M_Z * [(t_f - t_o) / 24]$$

where F_Z is the average daily frequency of changes of magnitude Z , M_Z is the number of pairs of transition points having a magnitude Z , t_f is the time of the end of the CGM data, t_o is the time of the start of the CGM data, and $(t_f - t_o)$ is a value expressed in hours.

[00156] The processor 120 then determines a weighted sum, the dynamic stress factor (DySF), of the magnitude of large transitions (block 168). In an embodiment, large transitions are transitions with a magnitude greater than one. That is, DySF is calculated according to the equation:

$$\text{DySF} = \sum F_Z * |Z|$$

for all values of $|Z| > 1$, where F_Z is the average daily frequency of changes of magnitude Z , and $|Z|$ is the magnitude of Z .

[00157] The following examples are given merely to illustrate the present invention and not in any way to limit its scope.

EXAMPLES

EXAMPLE 1

[00158] *Abstract*

[00159] Hemoglobin Ale (HbA1c) is the current standard used in the clinical treatment of patients with diabetes. However, it has been shown that patients with similar HbA1c values may have widely different fluctuations in blood glucose values over the same period of time, including time spent in hyper- and/or hypo-glycemia. Hence, there exists a need for quantitative

measures that can supplement HbA1c in managing patients with diabetes. We introduce and compare the Dynamic Stress Factor, DySF, a newly developed metric that quantifies glycemic volatility based on patient-specific glucose transition density profiles with HbA1c and with currently used glucose variability metrics in predicting severe hypoglycemia in children with type 1 diabetes. DySF, the daily weighted number of large monotonic glycemic transitions that occur within one hour, was calculated for 441 total subjects with type 1 diabetes (146 children, aged 8-14 yrs) to assess the magnitude and frequency of glucose transitions per day. Severe hypoglycemic episodes (HE) were quantified for all subjects and evaluated against HbA1c and existing measures of glucose variability, including SD, MAGE, MODD, and CONGA using logistic regression models. DySF was found to be a predictor of severe HE in children ($p = 0.018$) with the likelihood of a child, aged 8-14 yrs, experiencing severe hypoglycemia increasing by up to 20% with decreasing values of up to 60% of DySF. Patients of any age who had one or multiple severe hypoglycemic episodes had on average a lower DySF when compared to those with no HE. Additionally, when considering mean glucose levels, DySF/mean was a preliminary predictor of severe HE in patients with HbA1c $\leq 6.5\%$ ($p = 0.062$). DySF is a dynamic, quantitative, measure of daily glucose "volatility" that separates patients, within the same strata of HbA1c, into visually distinct patient profiles. DySF can be used as a preliminary predictor of clinically severe hypoglycemia in children and "well-controlled" patients with HbA1c $\leq 6.5\%$.

[00160] *Introduction*

[00161] We have previously reported the development of the CGMGUIDE [19], an easy-to-use tool, that provides researchers and clinicians with a superior assessment of a patient's glucose landscape. The interface calculates and displays multiple metrics from inputted CGM data, offering not only a multifaceted approach to studying glucose variability, but also a means to investigate variability with more information-rich data sets. Here, we report a new sensitive metric, Dynamic stress factor (DySF). DySF was developed with the CGM-GUIDE, which performs superiorly in quantifying glucose volatility by taking into account the speed and magnitude of glycemic excursions between clinically-defined states. DySF employs the recently developed transition density profile from CGM-GUIDE® [19], which analyzes glucose excursions and transitions across different glycemic ranges, to predict the likelihood of onset of

severe hypoglycemic episodes in a cohort of patients with type 1 diabetes. Based on continuous glucose dynamics, DySF is thereby a measure of a patient's daily glucose "volatility".

[00162] *Research Design and Methods*

[00163] We analyzed publicly archived CGM data from the Juvenile Diabetes Research Foundation (JDRF) Continuous Glucose Monitoring Randomized Trial [20]. Trial protocol has been described previously in detail [20]. Briefly, the enrollment criteria were children and adults with type 1 diabetes mellitus (T1DM) for more than 1 year (aged 8 to 85 yrs), use of either an insulin pump or at least three daily insulin injections, and HbA1c < 10.0%. This analysis used CGM data and HbA1c levels collected at baseline from 441 T1DM patients with complete demographic and CGM data. Of the 441 patients analyzed, 32.4% were aged 8-14 yrs, 30.8% were aged 15-24 yrs and, 36.7% were ≥ 25 yrs. Patients were stratified into approximately equal percentages of subjects with HbA1c $\leq 7\%$ or $> 7\%$ but can easily be stratified into any percentage based on clinical advise. Hypoglycemia was defined as a glucose value of < 70 mg/dL. A severe hypoglycemic event was defined as an event requiring the assistance of another person to actively administer carbohydrate, glucagon, or other resuscitative actions in the presence of seizure or coma [20]. DySF is the daily weighted number of large monotonic glucose transitions that occur in less than one hour. DySF is derived from transition density profiles, described in [19], with the exception of employing equally-spaced bin thresholds for DySF analysis (Figure 1). First, raw CGM data were partitioned into 40 mg/dL bins and exact transition points were established (Figure 1a,b). Second, the magnitude of every continuous monotonic change in glucose bin levels was sorted into the number of thresholds crossed (e.g. 2, 4, -3, -5) (Figure 1c). Negative numbers indicate monotonic decreases and positive numbers indicate monotonic increases in glucose levels. Third, transitions were separated into the time interval necessary to complete each change (i.e. < 1 h, between 1-2 h, 2-3 h, etc.). Finally, the frequency of each monotonic threshold crossing per day was plotted against the time interval needed to cross the indicated number of thresholds (Figure 1d).

[00164] DySF calculation has been added to the analytical software CGMGUIDE © (patent-pending) as the weighted daily number of ($> |40|$ mg/dL) monotonic transitions that occur within one hour [19] (Figure 1d). To calculate DySF, a patient's raw CGM data is first analyzed by the transition density profile method outlined above, using glucose bin threshold intervals of 40

mg/dL. Monotonic transitions (i.e. periods of monotonic threshold crossings) that occur within one hour and that exceed a magnitude of 1 threshold (40 mg/dL) are identified. Each transition is assigned a magnitude equal to the number of thresholds crossed during that transition (For example, a monotonic decrease in glucose level across three thresholds would be given a magnitude of -3). The sum of the absolute value of these scaled transition magnitudes is then divided by 24 to give the DySF units of weighted number of transitions per day (Figure 1d). Robustness and sensitivity of DySF were evaluated for a range of choices in glucose threshold bin size (5 - 100 mg/dL) without observed improvement above the standard 40 mg/dL in correlation to HE. Additionally, bin sizes of 40 mg/dL correspond to clinically important glycemic boundaries. The maximum glucose sampling interval at which DySF could be consistently measured was evaluated as <10 minutes using pair-wise t-tests for 1 min, 5 min, 10 min, 15 min, and 30 min intervals. A logistic regression model was used to predict the likelihood of observing a severe hypoglycemic episode in the 6 months prior to CGM monitoring. Covariates considered were mean glucose, standard deviation of mean glucose, standard deviation of transition speeds, DySF, MAGE, MODD, and CONGA [1].

[00165] Results

[00166] DySF as an indicator of Severe Hypoglycemia in children with type 1 diabetes

[00167] The demographics and other clinical characteristics of this cohort were published in [20]. DySF was calculated at baseline for 441 T1DM patients where 53.02% were female, mean age was 24.6 years, and mean HbA1c was 7.4%. These patients had an average DySF of 7.14 ± 5.39 with a cohort minimum at 0.33 and maximum at 54.89. Patients grouped by age and HbA1c had an average DySF of 7.00 ± 3.90 (age 8-14), 8.78 ± 7.50 (age 15-24), 5.88 ± 3.91 (age ≥ 25), 5.88 ± 5.63 (HbA1c ≤ 7), and 7.78 ± 5.20 (HbA1c > 7). In addition, to assess overall glycemic variability, CGM-GUIDE profiles were created for all patients to calculate the most widely used glycemic metrics and statistics discussed in Methods. A representative CGM-GUIDE profile that includes most widely used glycemic metrics and statistics is shown in Figure 2a.

[00168] To predict the occurrence of hypoglycemic episodes, simple and multiple logistic regression models were fitted to the data and all glycemic metrics were compared (Figure 2 a-c). Among the T1DM patients analyzed who had a mean glucose ≤ 140 mg/dL at baseline, DySF divided by the mean glucose (DySF/mean) was the best predictor of the frequency of

hypoglycemic episodes (p-value = 0.13) (Figure 2b). The ratio DySF/mean was also the best predictor of frequency of hypoglycemic episodes in T1DM patients with a baseline HbA1c $\leq$ 6.5% (p-value = 0.06) and in children aged 8-14 years (p-value = 0.018) (Figure 2b). Correlation between DySF and HbA1c, mean glucose, and other glycemic variability metrics showed low overlap of information between metrics (Figure 2c). Pearson correlation between DySF and each of the other measures of glycemic variability demonstrated that DySF provided additional information about glycemic variability with the exception of CONGA and standard deviation of slopes (Figure 2c). We also found that in this cohort of T1DM patients, individuals within the same level of HbA1c values demonstrated widely different DySFs, or volatilities, indicating patient-specific HbA1c-independent variations in glucose transition times and/or dynamic ranges. In addition, individuals who exhibited similar HbA1c levels (including individuals with HbA1c below 7.0%) had highly variable glucose volatility as measured by variable DySF. Individuals with the higher DySF values were those with poorer glycemic control as documented by HbA1c values larger than 7% (Figure 3). When comparing patients of any age who had zero, one, or multiple severe hypoglycemic episodes, average DySF values decreased incrementally with increased incidence of severe HE, 7.27 ± 5.6 , 6.63 ± 4.23 , 5.61 ± 3.47 , respectively.

[00169] Based on DySF values, a logistic model was used to predict the likelihood of children, 8-14, experiencing severe hypoglycemia. Children in the study who had the lowest DySF values (close to zero) had a 20% higher probability of having at least one severe hypoglycemic episode that required the assistance of another person for resuscitative actions (data not shown).

[00170] *Conclusion*

[00171] DySF is a new metric for the measurement of glycemic variability that measures the volatility of a patient's glucose dynamics by weighting the daily average of glucose transitions that occur in less than one hour. Using logistic regression models, DySF was found to be the most significant predictor of severe hypoglycemic episodes in children aged 8-14 years old, in patients with mean glucose less than or equal to 140 mg/dL (Figure 2b) and in patients with HbA1c $<$ 6.5%. Lower DySF values corresponded to higher risk of hypoglycemia and were indicative of smaller and/or slower glucose transitions over time. Several insights can be drawn from these results. First, we found that DySF/mean is a sensitive tool that can more favorably assess and predict patients' risk for experiencing severe hypoglycemic episodes compared to HbA1c alone.

We also confirm in a large sample of T1DM patients that low HbA1c levels can be misleading as an indicator of glycemic control. Patients with HbA1c below 6.5% are traditionally considered to have "well-controlled" diabetes [1]. We demonstrate in this cohort, that a lower HbA1c level (< 6.5%) may be the result of a high incidence of hypoglycemia as opposed to tighter glycemic control. We also show that by using DySF/mean, we are able to significantly enhance our ability to predict severe hypoglycemic events in patients with either low HbA1c, low mean BG and in children. This has very important clinical significance as it helps create a patient specific phenotype that can be used by clinicians to prevent severe hypoglycemic events. Second, the association between DySF and the occurrence of severe hypoglycemic episodes was found to be most significant in children (aged 8-14 years) across all levels of HbA1c (Figure 2b). Subjects were observed to vary widely in DySF values within the same class of HbA1c, suggesting the degree of glucose fluctuations to be independent of HbA1c level. This allows DySF to provide a different perspective on glycemic variability from what HbA1c measures—namely, volatility. Some studies have evaluated how children with T1DM identify severe hypoglycemic episodes [20], which is critical for their prevention. Gonder-Fredrick et al. demonstrated that children with type 1 diabetes failed to recognize greater than 40% of hypoglycemic occurrences, and Meltzer et al. observed that the average adolescent patient made irrelevant or inaccurate glucose estimations greater than 61% of the time [21,22]. Because HE often occur during times when patients fail to recognize symptoms associated with hypoglycemia, DySF can be used to assess the severity and speed of glycemic excursions and therefore can be an effective clinical tool to prevent HE and its serious consequences. Lastly, the correlation between DySF and other glycemic variability metrics is relatively low, with often less than 50% of the variation in DySF being accounted for by other metrics (Figure 2c). This suggests that a combination of existing glycemic variability metrics and DySF will be best suited to assess different populations and/or varying disease complications. A recent study by Guerra et al. explored rates of change of glucose, using a deconvolution algorithm that introduces uncertainty in the model parameters and considers less than 30 minutes of glucose history to predict future risk [23]. Previous glucose variability metrics, such as MODD, are considered to be a measure of daily glucose consistency [16], MAGE a measure of fluctuation severity, and CONGA a measure of glucose lability [16], or the likelihood of undergoing any change in glucose level over a defined length of time. By introducing DySF, a measure of glucose volatility, we can now explore over much longer

histories of BG data, the long-term effects of changes in glucose speed and magnitude on patient outcomes. DySF therefore increases significantly the predictive power for hypoglycemia and other complications. In addition, we demonstrate that DySF offers tailored information about specific populations, such as children (8-14 years), or patients with various HbA1c levels. Despite clear differences in their defining properties, to date existing glucose variability metrics are used either interchangeably or individually with statistics such as SD to assess overall glycemic variability. Cameron et al. demonstrated that glucose variability metrics, though correlated with each other in non-diabetic patients, are not correlated in diabetic populations [9]. Clarke and Kovatchev [24,25] have applied metrics for studying hypoglycemic events in patients using single monitor blood glucose (SMBG) measurements. Their studies show some predictive measures of future hypoglycemic events but the metrics were strongly correlated to the past history of time spent in lower glycemic ranges and did not consider the entire course of patient data which includes hypo and hyper regions as well as normal ranges. Thus, studies in diabetic populations that look at only one or two measures, or less sensitive measures of glucose variability, cannot comprehensively assess glycemic variability because these do not take into account the full range of glycemic states a patient may encounter over shorter or longer periods of time, nor the spectrum of transition profiles from these states [26-28], whereas glucose variability profiles such as those generated by CGM-GUIDE overcome this challenge. DySF and CGM-GUIDE profiles may also prove to be superior to current individual metrics in evaluating the role of glucose variability in the development of chronic diabetes complications [19]. At present, low DySF is important in assessing trends in hypoglycemia; however, high volatility may become important when assessing chronic diabetes complications and disease progression. Rapid glucose fluctuations have been hypothesized to incorporate "stress" into a patient's system by increasing oxidative stress and contributing to the development of microvascular complications and cardiovascular disease [3-5,8,29,30]. However, others have questioned this concept [31]. The long-term effect of sustained volatility is the next pressing question in developing an improved picture of diabetes progression toward chronic conditions, especially in what are currently considered well-controlled populations. Towards this effort, researchers and clinicians are now able to apply DySF, in conjunction with HbA1c, as a tool to enhance their ability to understand type 1 diabetes and to procure treatment options.

EXAMPLE 2

[00172] This example provides a simple example of how to calculate DySF.

[00173] In exemplary embodiments, DySF is the daily weighted number of large monotonic glycemic transitions that occur within one hour. The horizontal lines on the graph of Figure 13 de-mark the separate 40 mg/dL bins. Each tick mark on the X-axis represents 5 minutes. The binned glucose data are shown in Figure 14. The next step (see Figure 15) is to identify monotonic transitions (glycemic transitions) and determine the time interval (duration) for each one. The next step is to calculate the magnitude of monotonic transitions. See Figure 16. Next, add the relevant transitions. In this example, the properties of a "relevant" transition is (1) having a transition time interval which is < 1 hour and (2) having a transition magnitude of $>$ the **11**. See Figure 17. If a transition contains a gap, then the appropriate time interval is added to the transition, if the gap was < 15 min, or the transition is considered invalid, and is not used in the calculation of DySF, if the gap was > 15 min. See, Figure 18. The calculation of DySF with gaps is shown in Figure 19.

EXAMPLE 3

[00174] Abstract

[00175] **OBJECTIVE:** To compare the Dynamic Stress Factor (DySF), a newly reported metric that quantifies glycemic volatility based on patient-specific transition density profiles, with the hemoglobin A1c (HbA1c) and with currently used glucose variability metrics in predicting severe hypoglycemia in children with type 1 diabetes.

[00176] **RESEARCH DESIGN AND METHODS:** DySF, the daily weighted number of large monotonic glycemic transitions that occur within one hour, was calculated for 441 total subjects with type 1 diabetes (146 children 8-14 yrs) to assess the magnitude and frequency of glucose transitions per day. Severe hypoglycemic episodes (HE) were quantified for all subjects and evaluated against existing measures of glucose variability, including HbA1c, SD, MAGE, MODD, and CONGA using logistic regression models.

[00177] **RESULTS:** DySF was found to be a predictor of severe HE in children ($p = 0.018$) with the likelihood of a child, 8-14 yrs, experiencing severe hypoglycemia increasing by up to 20% with decreasing values of DySF. Similarly, patients of any age who had one or multiple severe hypoglycemic episodes had on average a lower DySF when compared to those with no

HE. Additionally, DySF/Mean was a preliminary predictor of severe HE in patients with HbA1c < 6.5% ($p = 0.062$).

[00178] **CONCLUSIONS:** DySF is a quantitative measure of daily glucose "volatility" that separates patients, within the same strata of HbA1c, into visually distinct patient profiles. DySF can be used as a preliminary predictor of clinically severe hypoglycemia in children and "well-controlled" patients with HbA1c < 6.5%.

EXAMPLE 4

[00179] *Abstract*

[00180] **Background:** Numerous metrics exist with the goal of linking glucose variability to diabetic complications, but an integrated approach is necessary to provide the most complete and consistent assessment of glycemic variation. Many investigators, however, have not readily or adequately adopted the use of multiple glucose variability metrics to evaluate glycemic variation due to the sometimes difficult or tedious coding necessary during quantification.

[00181] **Methods:** We compiled the most extensively used statistical techniques and glucose variability metrics with an intuitive means of entering patient data, adjusting hyper- and hypoglycemic limits, and metric parameters to create a user-friendly Continuous Glucose Monitoring Graphical User Interface for Diabetes Evaluation (CGM-GUIDE). In addition, we introduce and demonstrate a novel *transition density profile* that emphasizes the dynamics of transitions between defined glucose states.

[00182] **Results:** Our combined dashboard of numerical statistics and graphical plots support the task of providing an integrated approach to describing glycemic variability, including existing metrics such as, standard deviation (SD), area under the curve, and the mean amplitude of glycemic excursion (MAGE), but also novel metrics such as the slopes across critical transitions and the *transition density profile* to assess the severity and frequency of glucose transitions per day as they move between critical glycemic zones.

[00183] **Conclusion:** By presenting the above mentioned metrics and graphics in an aggregate, concise format, CGM-GUIDE provides an easy to use tool to compare quantitative measures of glucose variability and glean further insight into the connection between glucose variability, insulin delivery and clinical complications associated with diabetes.

[00184] *Introduction*

[00185] As the use of continuous glucose monitors (CGMs) becomes more prevalent in the management of diabetes, metrics for interpreting and connecting CGM data to therapeutic algorithms of insulin delivery are essential for the most effective use of CGMs in clinical care, with the ultimate goal of preventing chronic diabetes complications including nephropathy and kidney failure, retinopathy and blindness, peripheral neuropathy, and cardiovascular disease. Glucose variability is under consideration as a possible link to diabetic complications with studies reporting its role in promoting increased oxidative stress ¹ and vascular pathology.^{2,3} Currently, the statistics and metrics employed to reflect glucose dynamics include, but are not limited to, the overall standard deviation (SD) from a mean glucose value, percentage of values within, above, or below specified thresholds, area under the curve, mean amplitude of glycemic excursion (MAGE) ⁴, mean of daily differences (MODD) ⁵, and continuous overall net glycemic action (CONGA(n)). ⁶ Additional metrics include the M-value ⁷, average daily risk range (ADRR) ⁸, GRADE scores ⁹ and J-index.¹⁰ Inconsistencies, however, can arise from the miscalculation, misinterpretation, and misuse of these metrics in disparate data types and applications.

[00186] Of primary concern is the practical implementation of rigorous glucose variability metrics and glycemic statistics in the clinic, where there is currently a lack of software that provides easy quantification and comparison of glucose variability data. Here we provide a standardized approach for visualizing the dynamics associated with crossing into different regions of glycemia through the development of a novel histogram plot—from here onwards referred to as the *transition density profile*—that assesses the severity and frequency of glucose transitions per day as they move between critical glycemic zones. Also, to address the translational gap between existing metrics and their actual employment in clinical practice, we have created the Continuous Glucose Monitoring Graphical User Interface for Diabetes Evaluation (CGM-GUIDE) to integrate the evaluation of CGM data.

[00187] *Materials and Methods*

[00188] CGM data were provided by the Pop-Busui Lab and were collected on an iPro CGM System (Medtronic, Northridge CA) in adult patients with type 1 diabetes (T1D) under normal daily conditions. Here we present data from one representative patient in detail (Patient3) to

appreciate the full functionality of CGM-GUIDE. Non-diabetic reference metric values were reported in independent studies by McDonnell et al.,⁶ based on CGM traces for 10 healthy non-diabetic controls, and Cameron et al.,¹¹ based on CGM traces for 12 healthy non-diabetic controls (Figure 5). Patients were sampled at 5-minute intervals for up to 140 hours. CGM-GUIDE was designed using Matlab Version 2008b with descriptions of user inputs and novel CGM-GUIDE outputs including transition speeds, and the *transition density profile*, as well as standard statistics and metrics:

[00189] Time Interval (min): Time interval at which CGM blood glucose (BG) data were collected.

[00190] Bin Thresholds (mg/dL): The bounding BG values into which raw CGM BG data will be binned, such that Bin(l) contains all the raw BG values that satisfy the condition $[\text{threshold}(l) < \text{BG} \leq \text{threshold}(2)]$ (Figures 7A-E).

[00191] Hyperglycemic / Hypoglycemic Limit (mg/dL): All BG values greater than the hyperglycemic limit and less than or equal to the hypoglycemic limit will be considered hyper- or hypoglycemic, respectively.

[00192] Time Hyperglycemic / Hypoglycemic (h): Estimation of total time spent in the hyper- or hypoglycemic condition. A linear model was used to approximate the time spent in the hyper- / hypoglycemic ranges during the threshold crossing. Glycemic time is expressed both in hours and as a percentage of the total CGM monitoring period.

[00193] Time Spent in Each Bin (h): Estimation of the total time spent in each bin (as defined above in "Bin Thresholds").

[00194] Mean Glucose (mg/dL): The arithmetic mean of all raw CGM BG values.

[00195] Standard Deviation (SD): The sample standard deviation of all raw CGM BG values.

[00196] Mean of Daily Differences (MODD): The mean of the absolute value differences between glucose concentrations measured at the same time of day on two consecutive days (Figure 6).

[00197] Continuous Overall Net Glycemic Action (CONGA(n)): The SD of the differences between a current observation and an observation (n) hours earlier (Figure 6).

[00198] Mean Amplitude of Glycemic Excursion (MAGE): The arithmetic average of absolute value differences between adjacent glucose peaks and nadirs, where the differences exceed 1 SD from the mean. Algorithm described in Fritzsche *et. al.*¹²

[00199] Area Under the Curve: Graphical representation of two sets of cumulative areas under the curve—a hyperglycemic set and a hypoglycemic set. The areas were computed using a trapezoidal numerical integration function.

[00200] Input Data Plot: Line plot of the raw CGM data. The horizontal lines indicate the user-defined glycemic thresholds (Fig. 7A,B).

[00201] Transition Speed: CGM-GUIDE provides two assessments of the slopes of patient CGM data: 1) a newly developed scatter plot of slopes at transition points (Fig. 7C), and 2) a histogram of the distribution of slopes between every recorded time interval (Fig. 7D). The histogram presents the distribution of slopes between every two consecutive glucose measurements in the patient CGM data; the standard deviation of the slope is also calculated as a potential indicator of the rate of glucose fluctuations. The scatter plot displays the slopes of points flanking transitions from one user-defined threshold into another (as described in Transition Density Profile).

[00202] Transition Density Profile: First, raw CGM data are partitioned into bins based on user-defined threshold values and exact transition points are established (Fig. 7B). Second, the magnitude of every continuous monotonic change in blood glucose levels, after smoothing, is sorted into the number of thresholds crossed (e.g. 2, 4, -3, -5). Negative numbers indicate monotonic decreases and positive numbers indicate monotonic increases in glucose levels (Fig. 7E). Third, transitions are separated into the time interval necessary to complete each change (i.e. < 1 h, between 1-2 h, 2-3 h, etc.). Finally, the frequency of each monotonic threshold crossing per day is plotted against the time interval needed to cross the indicated number of thresholds (Fig. 7F).

[00203] Results

[00204] Mean, SD, and Time in Glycemic States

[00205] To highlight the methodology behind integrated glycemic variability assessment, we provide information on a representative patient (Patient 3) that captures the capability of the

CGM-GUIDE. First, PatientB had a mean glucose concentration of 121.9 mg/dL, which is a 25.76% increase above the average reference value for non-diabetic control patients (96.84 mg/dL), and a standard deviation of 55.2 mg/dL, notably 3-fold higher than the average standard deviation of non-diabetic controls (14.13 mg/dL) (Figure 5).

[00206] Since critical limits used to assess glucose control may be heterogeneously standardized for different applications, to accommodate for non-uniformity, we allow variable thresholding of the CGM data and calculate the time spent within each user-defined range. We chose threshold limits of (0, 50, 70, 180, 220, and 300) and observed Patient3 within these ranges 9.7%, 12.4%, 62.3%, 10.6%, 5.1% of the time, respectively (Fig. 7B). Additionally, we calculated that Patient3 experienced hyper- / hypoglycemia 15.6% and 22.0% of the monitored time, respectively, while the non-diabetic controls were never hyperglycemic, and were hypoglycemic an average of 6.12% of the time (Figure 5).

[00207] *MODD, CONGA(n), and MAGE*

[00208] As a measure of inter-day blood glucose variation, we calculated the mean absolute value of daily differences (MODD) between glucose concentrations (Figure 6). In Patient3, the average daily variation was 57.5 mg/dL, as compared to an average non-diabetic MODD value of 14.41 mg/dL. Due to inter-day comparisons, MODD is dependent on patients' adherence to a regular meal and insulin schedule. Clinical experience has indicated that CGM traces with high MODD values can be indicative of irregular habits and thus require detailed contemporaneous lifestyle information prior to interpretation (Figure 5).⁶

[00209] As a supplement to MODD, we analyzed the differences in blood glucose over a fixed interval using CONGA(n), which calculates the SD of glucose differences n hours apart. Due to the flexibility of CONGA(n), we can assess time increments shorter than 24 hours to provide a measure of intra-day glycemic variation and reduce dependence on the rigorous tracking of patient habits. CONGA(1), CONGA(2), and CONGA(4) for Patient3 were found to be 35.7, 54.8, and 71.5 mg/dL, respectively. These values are 2 to 4-fold greater than the corresponding mean non-diabetic control values of 12.91, 15.89, and 18.26 mg/dL, respectively (Figure 5). For healthy controls, the time period, n , used to calculate CONGA has minimal effect on the metric value. For diabetic patients, however, CONGA(n) values have been shown to increase with n (1 to 8 hours), gradually leveling off as n approaches 4 hours.¹¹

[00210] MAGE considers adjacent glucose peak and nadirs whose absolute differences exceed one standard deviation from the mean, and calculates the arithmetic average of these differences. Patients with unstable glucose concentrations are therefore expected to have higher MAGE values than normal, healthy individuals. Patient3 was found to have a MAGE value of 90.1 mg/dL, 3-fold greater than the average MAGE of 32.14 mg/dL found in healthy controls (Figure 5).

[00211] *Area Under the Curve, Slope Histogram, and Transition Speed Scatterplot*

[00212] For a more visual interpretation of blood glucose control, area under the curve plots depict the degree of glucose deviation above or below glycemic limits. In Patient3, the area above the hyperglycemic limit (180 mg/dL) was calculated to be 41220 mg*min/dL, whereas the area below the hypoglycemic limit (70 mg/dL) was 31315 mg*min/dL (Fig. 7C). Similar to mean CGM glucose values, a positive relationship has been shown between HbA1c and area under the curve values.¹³

[00213] Blood glucose rates of change offer a direct way to quantify and analyze the severity of fluctuations in blood glucose levels. CGM-GUIDE calculates the rate of change between every two consecutive CGM measurements, then consolidates all the slopes and presents them in a histogram (Figure 7D-insert). The standard deviation of the slope distribution is representative of the dispersion in speed of glucose changes and was measured as 1.21 mg/(dL*min) for Patient3. Notably, this standard deviation of slopes is distinct from the SD measured previously, which is a measurement of the variation in glucose concentrations.

[00214] In contrast to the slope histogram, which displays glucose rates of change calculated at every sampling point, the transition speed scatterplot focuses on glucose dynamics during threshold crossings. CGM-GUIDE calculates the blood glucose rate of change at the time of each threshold intersection and then plots the slope against the intersection time. Expressing these "transition speeds" in a graphical manner allows for ready visualization of dangerous glucose excursions that could be addressed by adjusting insulin therapy.

[00215] *Transition Density Profiles*

[00216] We developed a novel *transition density profile* that reports the frequency, relative magnitude, and time taken for blood glucose levels to cross user-defined thresholds. Transition density profiles permit easy assessment of glucose dynamics across critical thresholds of

glycemia (for calculation see the Materials and Methods section). Patient3's transition density profile depicted multiple large monotonic changes in glucose values (Fig. 7F). Large changes occurring over shorter timeframes are assumed to cause greater stress to the body than similar transitions occurring over longer timeframes. For example, the (-2 level) transitions that required between 1-2 h to complete would not cause the same level of stress as the more rapid (-2 level) transitions observed in less than 1 h (Fig. 7F).

[00217] *Safeguards and Compatibility*

[00218] The data collection time interval entered by the user is checked against published statistical limits within which variability metrics can be accurately assessed. When an interval exceeds limits, an error message states, "Data collected at intervals greater than 1 hour affect the accuracy of MAGE, and intervals greater than 2-4 hours affect the accuracy of SD and CONGA(n) where n=1, 2, or 4." ¹⁴ Non-continuous or missing data is also accounted for in metric calculations and graphs.

[00219] Data from the most popular CGM collection monitors and software systems including Freestyle Navigator CoPilot Health Management System (Abbott), CareLink Personal Therapy Management (Medtronic), MiniMed Solutions Pumps (Medtronic), and Dexcom Data Manager 3 (DM3) SEVEN PLUS (Dexcom) can be adapted for use with CGM-GUIDE.

[00220] *Discussion*

[00221] We present here a clinician-friendly CGM-GUIDE that simultaneously calculates statistics, including mean glucose and (percent) time spent in hyperglycemic and hypoglycemic conditions, along with data thresholding based on user-specified blood glucose ranges (Fig. 8). Thresholding allows for any range of blood glucose concentrations to be independently assessed for time spent in these ranges and for transition speeds between ranges to be estimated. Uniquely, CGM-GUIDE calculates the most widely used glucose variability metrics—standard deviation (SD), MODD, CONGA(n), and MAGE—and presents them visually all in one setting, like a dashboard. The aggregated format provides a more complete picture of glycemic control while facilitating comparisons between glucose variability and various insulin algorithms for the potential design of more accurate insulin adjustments. Additionally, CGM-GUIDE provides interactive graphical representations of CGM data including: 1) raw data with a display of user-defined threshold ranges, 2) area under the curve above or below user-defined hyper- and

hypoglycemic limits, 3) a new graph of transition speeds across user-defined thresholds, 4) a histogram of slopes, and 5) a novel histogram plot—the *transition density profile*—indicating the magnitude and frequency of monotonic increases or decreases in the glucose data, over the time duration necessary for transitions to occur.

[00222] Continuous glucose monitors have afforded patients and physicians the flexibility to track glucose trends throughout the day, to assess individualized response to exercise and various stressors, and to evaluate nocturnal blood glucose trends including frequency and trends of hypoglycemia. As the use of CGMs increases, conclusions previously drawn from finger prick profiles can be tested against this more robust data set and used to guide optimization in individualized insulin regimens and effective prevention of severe hypo and hyperglycemia. Ultimately data obtained with this tool may be used to assess the relationship with chronic diabetes complications and disease progression in either future long-term prospective clinical studies and/or with data previously collected during former follow-up studies. Cameron et al.¹¹ compared the performance of many of the metrics listed above and identified inherent properties and applications associated with each metric. Some of the metrics, such as ADRR, are more appropriate for routine self-monitored blood glucose (SMBG) data,⁹ whereas CONGA(n) was designed specifically for CGM data.¹⁵ Apart from the necessity to differentiate between methods for analyzing SMBG versus CGM readings, many investigators acknowledge the subjectivity of certain extant glucose variability metrics. For example, researchers have criticized the M-value and MAGE—two of the earliest glucose variability measurements formulated—for their reliance on glucose reference points and subjective definitions for glycemic peaks and nadirs.¹⁵ Nonetheless, MAGE is still one of the most commonly used metrics for describing glucose variability in diabetes studies. Only as of 2011, has any standardized computer algorithm been available for the calculation of MAGE.^{12, 16}

[00223] Rapid variability in glycemia has been thought to incorporate additional "stress" to a patient's system and has been reported to induce increased oxidative stress¹ and these in concert were proposed to contribute to the development of microvascular complications.^{2, 3} There exist, however, conflicting conclusions on the relationship between glucose variability and these aforementioned complications.^{2, 17} For example, some statistical models developed using the glycemic profile data collected during the landmark Diabetes Control and Complications Trial (DCCT) suggested a relationship between fluctuations in blood glucose and the development of

retinopathy, nephropathy and neuropathy.¹⁵ However, recent independent analyses of DCCT glycemia data performed by the DCCT coordinating center have contradicted these findings and reported that the SD of blood glucose does not relate to the progression of microvascular complications in general.^{19, 20} Similarly, Siegelar *et al.* used SD and MAGE to evaluate the relationship between DCCT glucose data and complications, concluding that glucose variability as assessed by these measures does not contribute to the development of peripheral and autonomic neuropathy.¹⁹

[00224] The inconsistent results on the relationship between some measures of glucose dynamics and the development of specific diabetes complications reported by these studies may be however due to the paucity of glucose data as obtained from five or seven point single monitor glucose profiles. These discrete measures of blood glucose provide only a limited view of a patient's glucose dynamics over 24 hours, therefore limiting the amount of information available for analysis as compared to the study of CGM data. In addition, as discussed earlier, statistical measurements of SD do not provide a comprehensive assessment of glucose variability, especially when the data set contains a relatively sparse set of glucose measurements. In particular, recent work suggests the calculations of SD and CONGA(4) become unreliable when data measurements are taken more than 2 to 4 hours apart while MAGE becomes unreliable at observation intervals greater than 1 hour.¹⁴

[00225] Furthermore, different measures of glucose variability may be appropriate for assessing different physiological conditions. Many studies that employ an individual metric in isolation to correlate glucose variability and clinical complications may observe correlations but are not complete in their assessment of variability. Cameron *et al.* demonstrated that glucose variability metrics, though correlated with each other in non-diabetic patients, are not correlated in diabetic populations.¹¹ Thus, studies in diabetic populations, that look at only one or two measures of glucose variability are somewhat misleading because they do not take into account the range of available glycemic metrics.^{21, 22, 23}

[00226] In contrast to diabetic patients, analyses of critically ill non-diabetic adult and pediatric intensive care unit (ICU) patients have indicated correlations between glucose variability and mortality regardless of illness or severity.¹⁷ Glucose variability, as measured by SD, has been shown to be a significant predictor of mortality in the adult ICU's by three

independent groups^{24, 25, 26} and in two different pediatric ICUs,^{27, 28} suggesting a need for glucose control in non-diabetic patients. These findings, along with the observation that mortality was observed to significantly increase with glucose variability in different strata of mean glucose levels,²⁶ suggest that glucose variability is a predictor of mortality independent from mean glucose level. Consequently, the calculation and monitoring of glucose variability would be of added importance for the general care of adult and pediatric patients in ICUs.

[00227] In summary, the development of CGM-GUIDE provides researchers and clinicians with a superior assessment of a patient's glucose landscape. The interface calculates and displays multiple metrics from CGM data, offering not only a multifaceted approach to studying glucose variability, but also a means to investigate glucose variability using more information-rich data sets. By combining metrics into an aggregate tool, we anticipate the use of integrated glycemic variability assessments to potentially supplement the current measurements of HBA1c, inform insulin adjustments and assist in more global assessments of the relationship between glucose variability and the development of diabetes complications.

EXAMPLE 5

[00228] This example provides a comparison of DySF to other known metrics.

[00229] There are a variety of metrics used to assess the quality of glycemic control in diabetics. Some are designed to measure risk specific to hyperglycemia and hypoglycemia, some target glycemic variability, and others are aimed at the overall quality of glycemic control. Given these broad categories, one expects some glycemic control metrics to be clustered more closely than others; that is we expect there exist groups of metrics that are more similar to one another than to metrics in other groups.

[00230] To make this clustering notion more precise, we perform an exploratory factor analysis on 17 metrics from the published literature. The goal of factor analysis is to learn a small number of independent (orthogonal) latent (unobserved) factors that explain much of the correlation structure in a multivariate data set. These factors can be thought of as directions in the space of the data along which most of the observed variability occurs. As discussed below, just three factors are needed to explain 87% of the observed inter-subject variability on these 17 metrics.

[00231] To be a bit technical, we can think of the factors as specifying a low(3)-dimensional manifold in a high(17)-dimensional space near which most of the observed data points (subject metric scores) lie. This is analogous to a simple linear regression in which the data in 2-dimensional space are seen to lie near a 1-dimensional manifold (the regression line). However, unlike a regression in which Y is a noisy function of X both in a factor analysis both X and Y are noisy functions of third unobserved (latent) variable Z. See, Figure 20,

[00232] Using the JDRF trial data, we compute the 17 metrics for each of 443 subjects. These metrics are transformed using a maximum likelihood based Box-Cox power transform in order to improve normality of the marginal distributions. This is important as there is an underlying assumption of normality in the setup of a factor analysis. On the basis of Home's Parallel Analysis we choose to retain 3 factors.

[00233] The first factor primarily accounts for poor hyper glycemic control. Metrics that load strongly onto this factor include both direct measures of hyperglycemia (Mean, Time Hyper, AUC Hyper, HBGI) and Variability metrics' that are inflated by time spent in the hyperglycemic region (SD, MODD, MAGE, CONGA (4)). We might term the latter group the 'Hyper Variability' metrics and the former 'Direct Hyper' metrics. GRADE also loads strongly onto this factor and is more closely associated with the 'Direct Hyper' group.

[00234] In contrast, the second factor predominately accounts for poor hyperglycemic control. Loading primarily onto this factor are: Time Hypo, AUC Hypo, and LBGI.

[00235] Interestingly, this factor also helps to distinguish between the 'Direct Hyper' and 'Hyper Variability' groups. 'Direct Hyper' metrics load negatively onto this hypo factor while 'Hyper Variability' metrics have positive loadings. The magnitudes are comparable between groups though much smaller than the loadings onto the first hyper factor. (A note on loadings: the squares are more directly comparable and interpretable as they represent explained variances.)

[00236] The three metrics that load primarily on the third factor are DySF, MAG, and SD Slope. Consequently, we might think of this as the 'Rate of Change Variability' factor as each of these metrics depends on the first derivative of the glucose curve. Both MAG and SD Slope also load rather strongly on the first hyper factor and positively but less strongly onto the hypo

factor. This reflects the fact that variability metrics share some communality and are inflated by both hyper and hypoglycemic extremes.

[00237] In contrast, DySF loads solely onto this third 'Rate of Change Variability' factor. DySF is alone in loading onto a single factor (at the cutoff rate of 1% explained variance) though AUC Hyper and Time Hypo come extremely close to loading solely onto their respective factors.

[00238] For each factor, we also have a 'uniqueness' the portion of the variance unexplained by the three factors. The M-Value is not well accounted for by any of the three factors with 87% unique variance. At 42% DySF has the second largest unique variance while each of the AUC measures are slightly above 20% unique variance. None of the other metrics have more than about 7% uniqueness. The high uniquenesses of these metrics largely reflects the fact that their distributions are far from normal even following the Box-Cox transformation. For the AUC measures this is due to excessive skewness while DySF is both zero-inflated and highly skewed.

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[00239] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[00240] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted.

[00241] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range and each endpoint, unless otherwise indicated herein, and each separate value and endpoint is incorporated into the specification as if it were individually recited herein.

[00242] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[00243] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

WHAT IS CLAIMED:

1. A method implemented by a processor in a computer, the method comprising:
 - receiving a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_f , each data point comprising a blood glucose measurement value and a time value;
 - determining, via the processor, a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing;
 - determining, via the processor, for each pair of successive transition points, $(X_{i,i}, X_{2,i})$:
 - (1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ;
 - (2) an amount, t_i , of time elapsed between the pair of transition points; and
 - (3) whether the blood glucose level is increasing or decreasing between the pair of transition points;
 - determining, via the processor, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{\max}}$ and (ii) a value Y_i equal to Z ;
 - determining, via the processor, an average daily frequency, F_Z , of changes of each magnitude Z ; and
 - determining, via the processor, a weighted sum, $DySF$, of the magnitude of large transitions.
2. The method according to claim 1, wherein the time value associated with each data point is a time value relative to a start time of data collection.
3. The method according to claim 1, wherein the time value associated with each data point is an absolute time value indicating the time at which the data point was collected.

4. The method according to any one of claims 1 to 3, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ reflects a number of thresholds crossed between the transition points, each threshold corresponding to a multiple of a predetermined bin size.

5. The method according to any one of claims 1 to 4, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_i = (X_{2,i} / A) - (X_{1,i} / A)$$

where A is a predetermined bin size.

6. The method according to any one of claims 1 to 4, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_i = [(X_{2,i} / A) - \text{mod}(X_{2,i} / A)] - [(X_{1,i} / A) - \text{mod}(X_{1,i} / A)]$$

where A is a predetermined bin size.

7. The method according to any one of claims 5 or 6, wherein A is 40 mg/dL.

8. The method according to any one of claims 5 or 6, wherein A is between 10 and 60 mg/dL.

9. The method according to any one of claims 1 to 8, wherein the average daily frequency F_Z for each value of Z is determined according to the equation:

$$F_Z = M_Z * [(t_f - t_0) / 24]$$

where $(t_f - t_0)$ is a value expressed in hours.

10. The method according to any one of claims 1 to 9, wherein determining a weighted sum of the magnitude of large transitions comprises determining a weighted sum of the magnitude of transitions $Z > 1$.

11. The method according to any one of claims 1 to 10, wherein determining a weighted sum of the magnitude of large transitions comprises determining a weighted sum according to the equation:

$$DySF = \sum F_Z * |Z|$$

for all values of $|Z| > 1$.

12. The method according to any one of claims 1 to 11, wherein the value $t_{i_{\max}}$ is 60 minutes.

13. The method according to any one of claims 1 to 11, wherein the value $t_{i_{\max}}$ is between 30 minutes and 150 minutes.

14. The method according to any one of claims 1 to 13, wherein determining a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing comprises ignoring non-monotonicities that do not cross any of a plurality of threshold values.

15. A computer-readable storage medium having stored thereon machine-readable instructions executable by a processor, comprising:

instructions for causing the processor to receive a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_p , each data point comprising a blood glucose measurement value and a time value;

instructions for causing the processor to determine a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing;

instructions for causing the processor to determine, for each pair of successive transition points, $(X_{1,i}, X_{2,i})$:

(1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ;

(2) an amount, t_i , of time elapsed between the pair of transition points; and

(3) whether the blood glucose level is increasing or decreasing between the pair of transition points;

instructions for causing the processor to determine, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{\max}}$ and (ii) a value Y_i equal to Z ;

instructions for causing the processor to determine an average daily frequency, F_z , of changes of each magnitude Z ; and

instructions for causing the processor to determine a weighted sum, $DySF$, of the magnitude of large transitions.

16. The computer-readable storage medium according to claim 15, wherein the time value associated with each data point is a time value relative to a start time of data collection.

17. The computer-readable storage medium according to claim 15, wherein the time value associated with each data point is an absolute time value indicating the time at which the data point was collected.

18. The computer-readable storage medium according to any one of claims 15 to 17, wherein instructions for determining the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ include instructions for determining a number of thresholds crossed between the transition points, each threshold corresponding to a multiple of a predetermined bin size.

19. The computer-readable storage medium according to any one of claims 15 to 18, wherein instructions for determining the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ include instructions for determining the magnitude Y_i according to the equation:

$$Y_1 = (X_{2,i} / A) - (X_{1,i} / A)$$

where A is a predetermined bin size.

20. The computer-readable storage medium according to any one of claims 15 to 18, wherein instructions for determining the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ include instructions for determining the magnitude Y_i according to the equation:

$$Y_i = [(X_{2,i} / A) - \text{mod}(X_{2,i} / A)] - [(X_{1,i} / A) - \text{mod}(X_{1,i} / A)]$$

where A is a predetermined bin size.

21. The computer-readable storage medium according to any one of claims 19 or 20, wherein A is 40 mg/dL.

22. The computer-readable storage medium according to any one of claims 19 or 20, wherein A is between 10 and 60 mg/dL.

23. The computer-readable storage medium according to any one of claims 15 to 22, wherein instructions for determining the average daily frequency F_Z for each value of Z include instructions for determining the frequency F_Z according to the equation:

$$F_Z = M_Z * [(t_f - t_0) / 24]$$

where $(t_f - t_0)$ is a value expressed in hours.

24. The computer-readable storage medium according to any one of claims 15 to 23, wherein instructions for determining a weighted sum of the magnitude of large transitions comprises instructions for determining a weighted sum of the magnitude of transitions $Z > 1$.

25. The computer-readable storage medium according to any one of claims 15 to 24, wherein instructions for determining a weighted sum of the magnitude of large transitions comprises instructions for determining a weighted sum according to the equation:

$$DySF = \sum F_Z * |Z|$$

for all values of $|Z| > 1$.

26. The computer-readable storage medium according to any one of claims 15 to 25, wherein the value $t_{i_{max}}$ is 60 minutes.

27. The computer-readable storage medium according to any one of claims 15 to 25, wherein the value $t_{i_{max}}$ is 60 minutes.

28. The computer-readable storage medium according to any one of claims 15 to 27, wherein the instructions for causing the processor to determine determining a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing comprise instructions for ignoring non-monotonicities that do not cross any of a plurality of threshold values.

29. A system comprising:

a processor;

a memory device coupled to the processor, the memory device storing machine readable instructions that, when executed by the processor, cause the processor to:

receive a plurality of data points collected by a continuous glucose monitor between a first time t_0 and a second time t_f , each data point comprising a blood glucose measurement value and a time value;

determine a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing;

determine, for each pair of successive transition points, $(X_{1,i}, X_{2,i})$:

(1) a magnitude, Y_i , of the difference between the measurement values associated with the pair of transition points, the magnitude Y_i corresponding to one of a plurality of j integer values Z ;

(2) an amount, t_i , of time elapsed between the pair of transition points; and

(3) whether the blood glucose level is increasing or decreasing between the pair of transition points;

determine, for each of the plurality of integer values Z , the quantity M_Z of pairs having both (i) a value t_i less than a value $t_{i_{\max}}$ and (ii) a value Y_i equal to Z ;

determine an average daily frequency, F_Z , of changes of each magnitude Z ; and

determine a weighted sum, $DySF$, of the magnitude of large transitions.

30. The system according to claim 29, wherein the time value associated with each data point is a time value relative to a start time of data collection.

31. The system according to claim 29, wherein the time value associated with each data point is an absolute time value indicating the time at which the data point was collected.

32. The system according to any one of claims 29 to 31, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ reflects a number of thresholds crossed between the transition points, each threshold corresponding to a multiple of a predetermined bin size.

33. The system according to any one of claims 29 to 32, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_1 = (X_{2,i} / A) - (X_{1,i} / A)$$

where A is a predetermined bin size.

34. The system according to any one of claims 29 to 32, wherein the magnitude Y_i for a pair of points $(X_{1,i}, X_{2,i})$ is determined according to the equation:

$$Y_i = [(X_{2,i} / A) - \text{mod}(X_{2,i} / A)] - [(X_{1,i} / A) - \text{mod}(X_{1,i} / A)]$$

where A is a predetermined bin size.

35. The system according to any one of claims 33 or 34, wherein A is 40 mg/dL.

36. The system according to any one of claims 33 or 34, wherein A is between 10 and 60 mg/dL.

37. The system according to any one of claims 29 to 36, wherein the average daily frequency F_Z for each value of Z is determined according to the equation:

$$F_Z = M_Z * [(t_f - t_0) / 24]$$

where $(t_f - t_0)$ is a value expressed in hours.

38. The system according to any one of claims 29 to 37, wherein determining a weighted sum of the magnitude of large transitions comprises determining a weighted sum of the magnitude of transitions $Z > 1$.

39. The system according to any one of claims 29 to 38, wherein determining a weighted sum of the magnitude of large transitions comprises determining a weighted sum according to the equation:

$$DySF = \sum F_Z * |Z|$$

for all values of $|Z| > 1$.

40. The system according to any one of claims 29 to 39, wherein the value $t_{\max}$ is 60 minutes.

41. The system according to any one of claims 29 to 39, wherein the value $t_{\max}$ is 60 minutes.

42. The system according to any one of claims 29 to 41, wherein causing the processor to determine a plurality of transition points at which the blood glucose level changes from increasing to decreasing or from decreasing to increasing comprises causing the processor to ignore non-monotonicities that do not cross any of a plurality of threshold values.

43. The method, computer-readable storage medium, or system according to any one of claims 1 to 14, 15 to 28, or 29 to 42, respectively, wherein A is a first value for pairs of transition points in which both $X_{1,i}$ and $X_{2,i}$ are within a first range, and A is a second value for pairs of transition points in which $X_{1,i}$ and $X_{2,i}$ are not both within the first range.

44. The method, computer-readable storage medium, or system, respectively, according to claim 43, wherein the first range is about 70 mg/dL to about 120 mg/dL.

45. The method, computer-readable storage medium, or system, respectively, according to claim 43, wherein the first range is about 65 mg/dL to about 105 mg/dL.

46. A method of determining a subject's risk for a severe hypoglycemic episode, comprising the step of determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F, at which glycemic transitions of a magnitude, M, occur within a given time period, T, wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a

- negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and
 - c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1,

wherein, when the index of blood glucose volatility of the subject is decreased, as compared to a control index of blood glucose volatility, the subject is determined to be at risk for a severe hypoglycemic episode.

47. A method of determining a subject's need for prophylaxis of a severe hypoglycemic episode, comprising determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1,

wherein, when the index of blood glucose volatility of the subject is decreased, as compared to a control index of blood glucose volatility, the subject is determined to need prophylaxis of a severe hypoglycemic episode.

48. A method of preventing a severe hypoglycemic episode in a subject, comprising determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M ,
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1, and
- d. administering to the subject a prophylaxis of a severe hypoglycemic episode in an amount sufficient to prevent the severe hypoglycemic episode, when the index is decreased, as compared to a control index of blood glucose volatility.

49. A method of determining a subject's risk for a long term complication of diabetes, comprising the step of determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1,

wherein, when the index of blood glucose volatility of the subject is decreased, as compared to a control index of blood glucose volatility, the subject is determined to be at risk for a long term complication of diabetes.

50. A method of determining a subject's need for prophylaxis of a long term complication of diabetes, comprising determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,

- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1,

wherein, when the index of blood glucose volatility of the subject is decreased, as compared to a control index of blood glucose volatility, the subject is determined to need prophylaxis of a long term complication of diabetes.

51. A method of preventing a long term complication of diabetes in a subject, comprising determining an index of blood glucose volatility of a subject, wherein determining the index comprises the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M ,
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1, and
- d. administering to the subject a prophylaxis of a long term complication of diabetes in an amount sufficient to prevent the

long term complication of diabetes, when the index is decreased, as compared to a control index of blood glucose volatility.

52. A method of assessing an index of blood glucose volatility of a subject comprising the steps of

- a. analyzing blood glucose level data of the subject, which data was collected and recorded at short, regular intervals, for the frequency, F , at which glycemic transitions of a magnitude, M , occur within a given time period, T , wherein, when the glycemic transition is an increase in blood glucose levels, M is a positive integer and, when the glycemic transition is a decrease in blood glucose levels, M is a negative integer, wherein, for every unique M which is greater than a minimum magnitude threshold, MT_{min} , an F_M is determined,
- b. determining a weighted value, W_M , for every F_M , wherein each W_M is determined by multiplying F_M by the absolute value of M , and
- c. determining an index of blood glucose volatility by summing the values of each W_M for every M having an absolute value greater than 1,

wherein steps (a) through (c) are determined by the system of any one of claims 29 to 45.

53. The method of any one of claims 46 to 52, comprising collecting at short, regular intervals blood samples of the subject to determine blood glucose levels at each interval over a time period.

54. The method of any one of claims 46 to 52, wherein the blood glucose level data is obtained from a continuous glucose monitor (CGM).

55. The method of claim 549, wherein the short, regular intervals are X minutes apart, wherein X is an integer between about 0.5 and about 30.

56. The method of claim 55, wherein X is between about 5 and about 15.

57. The method of any one of claims 46 to 56, comprising implanting a CGM in the subject and obtaining data from the CGM to obtain blood glucose level data of the subject.

58. The method of any one of claims 46 to 57, excluding claim 52, wherein steps (a) through (c) are determined by the system of any one of claims 63 to 79.

59. The method of any one of claims 46 to 58, excluding claim 48, comprising administering to the subject a prophylaxis of a severe hypoglycemic episode, when the index is decreased, as compared to a control index of blood glucose volatility.

60. The method of any one of claims 46 to 59, excluding claim 51, comprising administering to the subject a prophylaxis of a long term complication of diabetes, when the index is decreased, as compared to a control index of blood glucose volatility.

61. The method of any one of claims 46 to 60, wherein the control index of blood glucose volatility is an index of blood glucose volatility of a population of subjects known to not suffer from a severe hypoglycemic episode or a long term complication of diabetes.

62. The method of claim 54, wherein the control index is an index of a population of subjects known to not suffer from diabetes or other metabolic disease.

63. The method of any one of claims 46 to 62, wherein the subject suffers from a metabolic disease.

64. The method of any claim 63, wherein the metabolic disease is diabetes, optionally, Type I Diabetes.

65. The method of any one of claims 46 to 64, wherein the subject is aged about 14 years or less.

66. The method of claim 65, wherein the subject is aged between about 8 and about 14 years.

67. The method of any one of claims 46 to 66, wherein the subject exhibits (i) a mean HbA1c level below 6.5 or (ii) a mean glucose level of about 140 mg/dL or below.

68. The method of any one of claims 46 to 67, further comprising determining one or more of the following of the subject: a mean of daily differences (MODD), a continuous overall net glycemic action (CONGA(n)), a mean amplitude of glycemic excursion (MAGE), an M-value, an average daily risk range (ADRR), a level of HbA1c, a point-error grid analysis (P-EGA), a Rate-error grid analysis (R-EGA), or a continuous glucose error grid analysis (CG-EGA).

69. The method of any one of claims 46 to 68, wherein the index of blood glucose volatility accounts for only glycemic transitions that occur within a range of durations.

70. The method of claim 69, wherein the index of blood glucose volatility accounts for only glycemic transitions that occur within about 1 hour.

71. The method of any one of claims 46 to 70, wherein T is about 24 hours or more.

72. The method of any one of claims 46 to 71, wherein MT_{m_h} is between about 10 and about 60 mg/dL.

73. The method of any one of claims 46 to 72, wherein MT_{min} is between about 25 and about 50 mg/dL.

74. The method of any one of claims 46 to 73, wherein MT_{mih} is about 40 mg/dL.

75. The method of any one of claims 46 to 74, wherein the index of blood glucose volatility is dynamic stress factor (DySF), as described herein.

76. The method of any one of claims 46 to 48 and 53 to 75, wherein the severe hypoglycemic episode is an occurrence of a blood glucose level about 70 mg/dL or less.

77. The method of claim 76, wherein the severe hypoglycemic episode is an occurrence of a blood glucose level about 70 mg/dL or less with one or more of the following physiological manifestations thereof: fainting, light headedness, dizziness, headache, seizure, coma, shock.

78. The method of any one of claims 46 to 77, comprising re-determining the index of blood glucose volatility of the subject after a therapeutic or prophylactic agent has been administered to the subject.

79. The method of any one of claims 46 to 78, comprising determining the index of blood glucose volatility of the subject before and after a test agent has been administered to the subject, wherein the efficacy of the test agent as a prophylaxis of a severe hypoglycemic episode or of a long term complication of diabetes is determined.

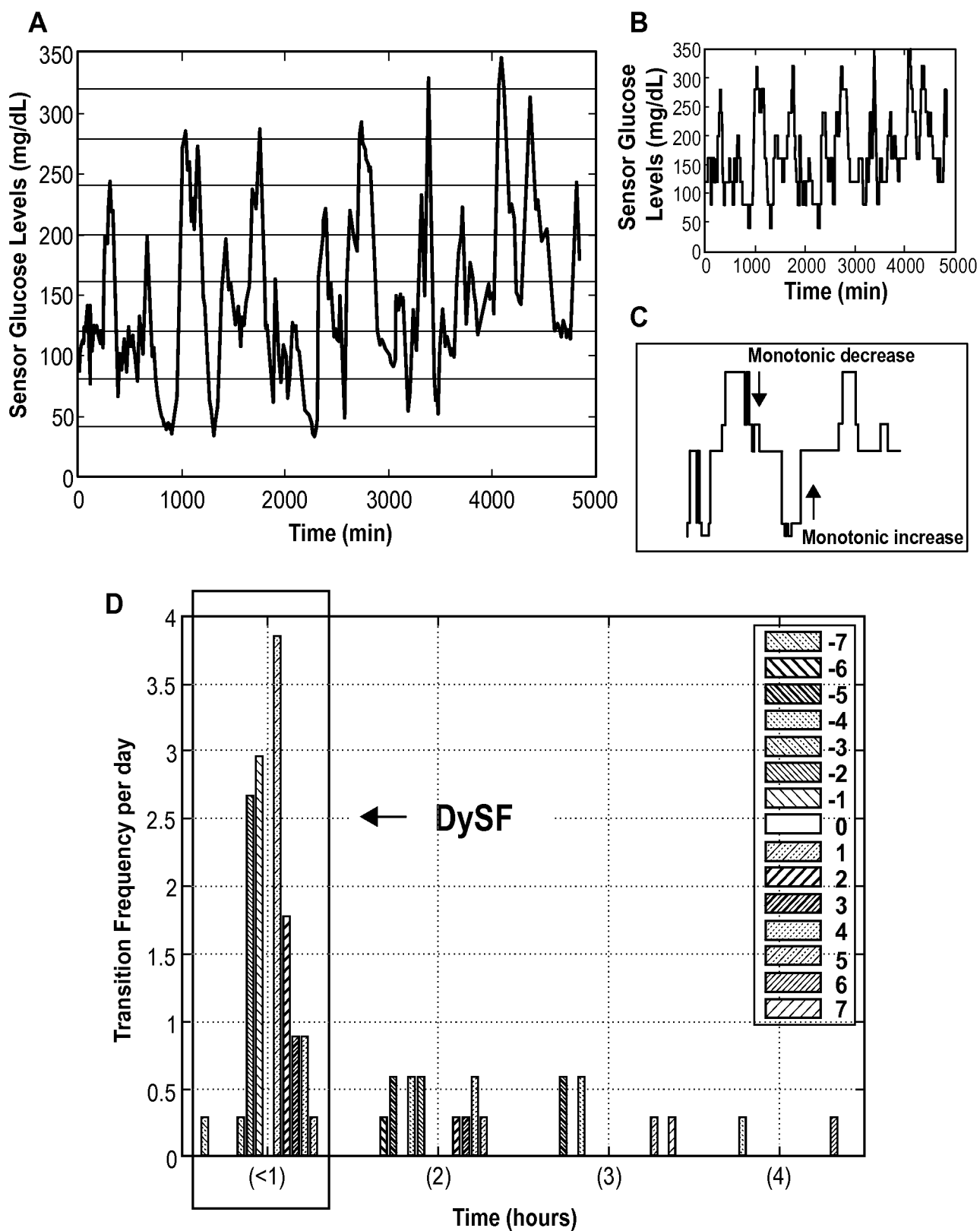


Figure 1

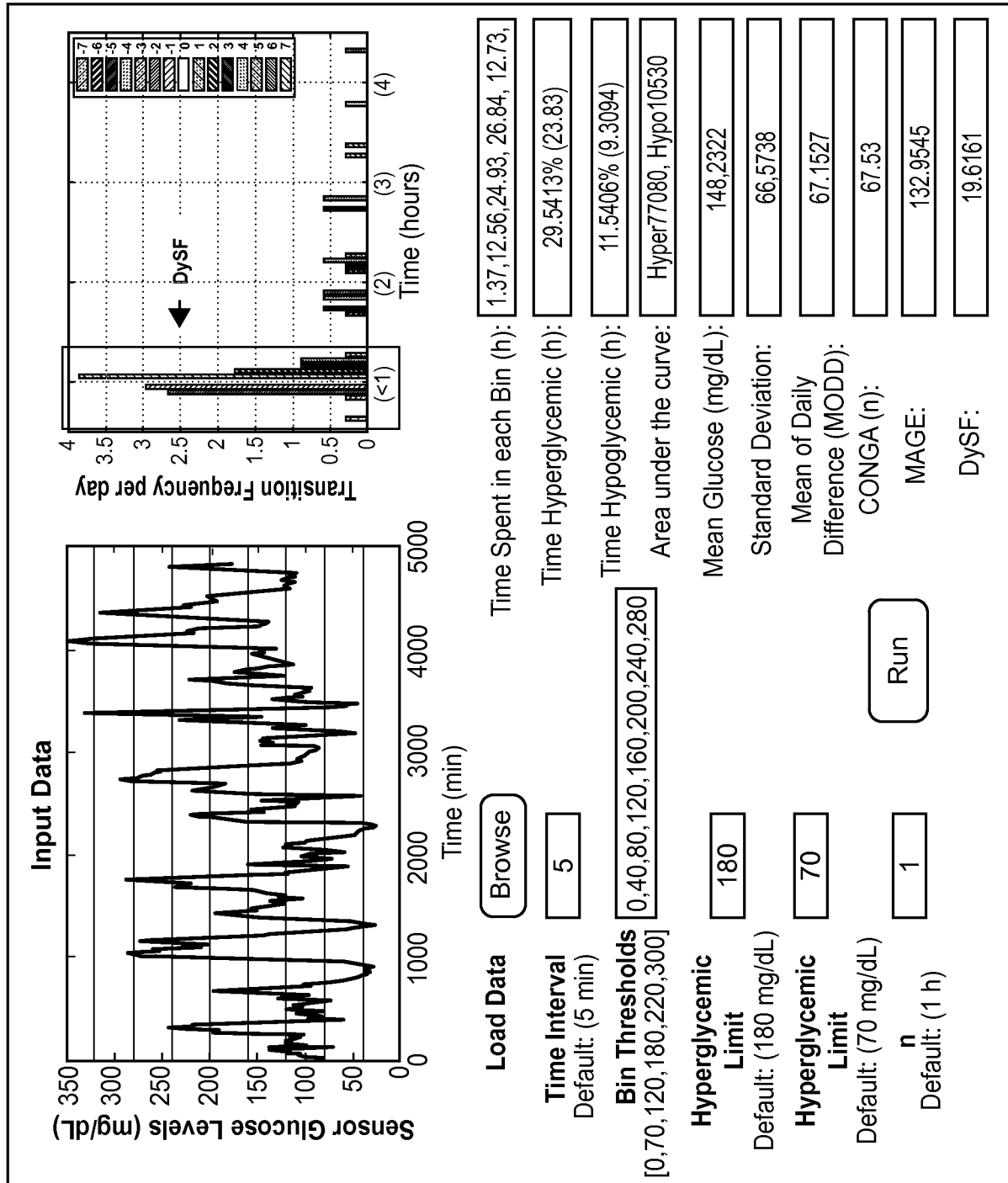


Figure 2A

Logistic Regression p-values by Group

		DySF	DySF/ Mean	MODD	CONGA (1)	HbA1c	Mean Glucose	SD	SD slope	MAGE	Sample Size
Age											
	(8-14)	0.018	0.032	0.388	0.261	0.075	0.079	0.078	0.148	0.252	143
	(15-24)	0.726	0.859	0.504	0.977	0.779	0.818	0.715	0.661	0.747	136
	(≥25)	0.373	0.098	0.965	0.293	0.553	0.092	0.579	0.584	0.539	162
Mean											
	(≤140)	0.279	0.135	0.949	0.161	0.945	0.218	0.367	0.608	0.311	93
	(140-180)	0.360	0.425	0.025	0.594	0.889	0.953	0.067	0.737	0.008	213
	(>180)	0.961	0.841	0.709	0.496	0.198	0.468	0.781	0.330	0.312	136
HbA1c											
	(1-6.5)	0.075	0.062	0.860	0.136	1.000	0.954	0.621	0.423	0.353	61
	(6.5-7.5)	0.968	0.891	0.091	0.445	1.000	0.704	0.058	0.649	0.033	188
	(>7.5)	0.088	0.478	0.737	0.107	1.000	0.005	0.285	0.226	0.286	191

Figure 2B

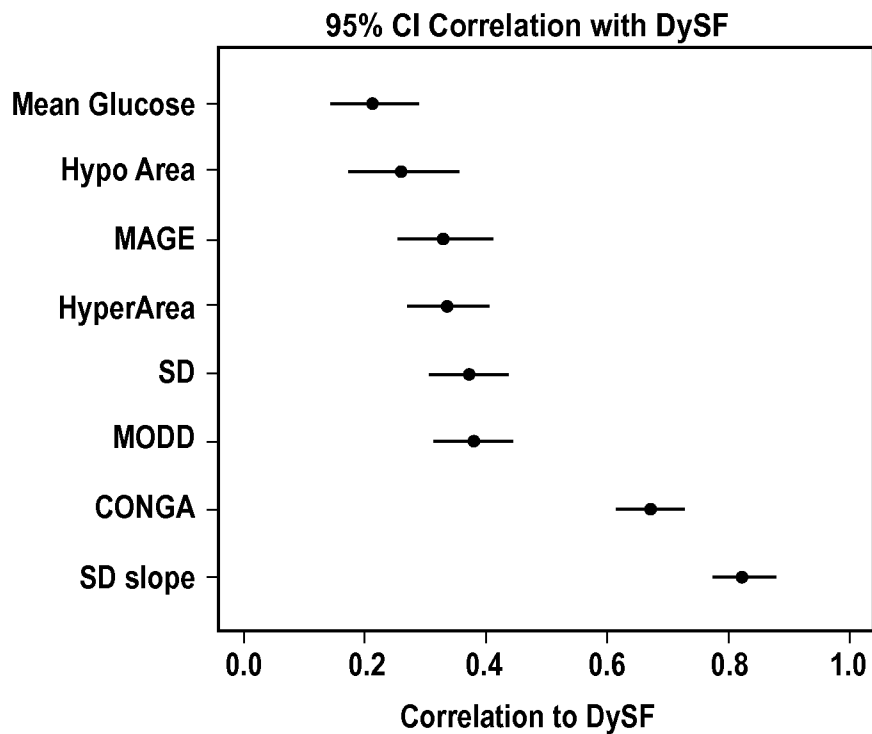
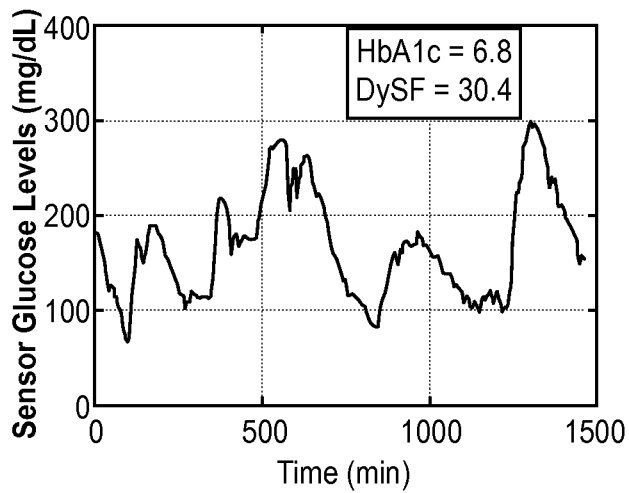
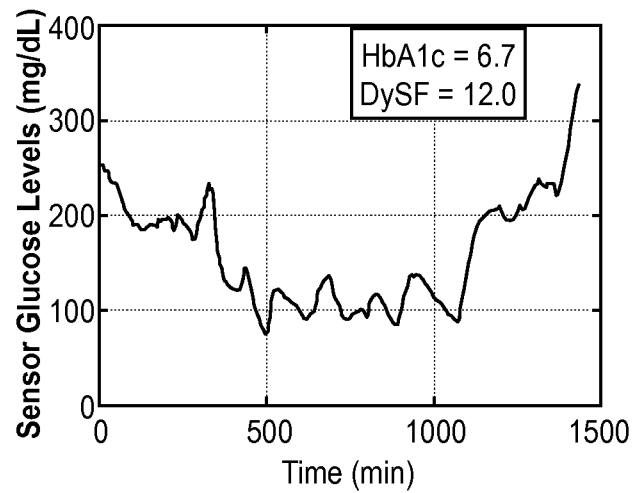
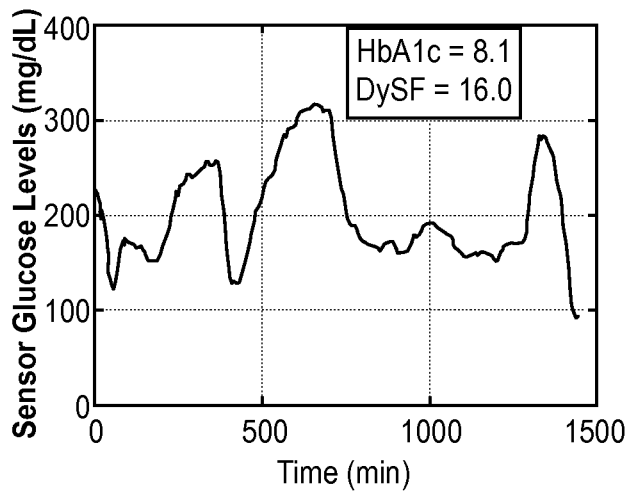
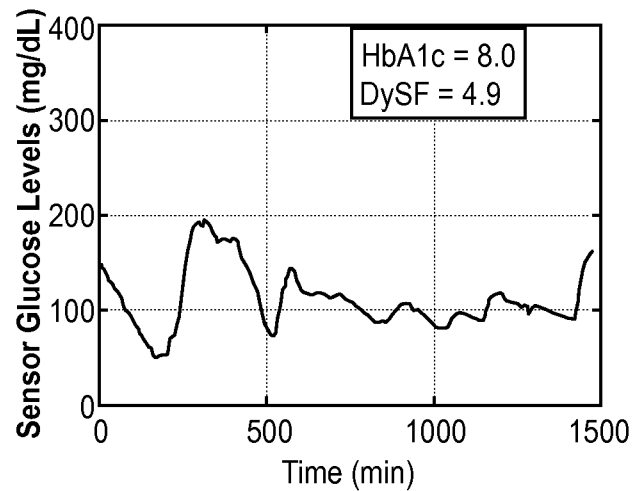
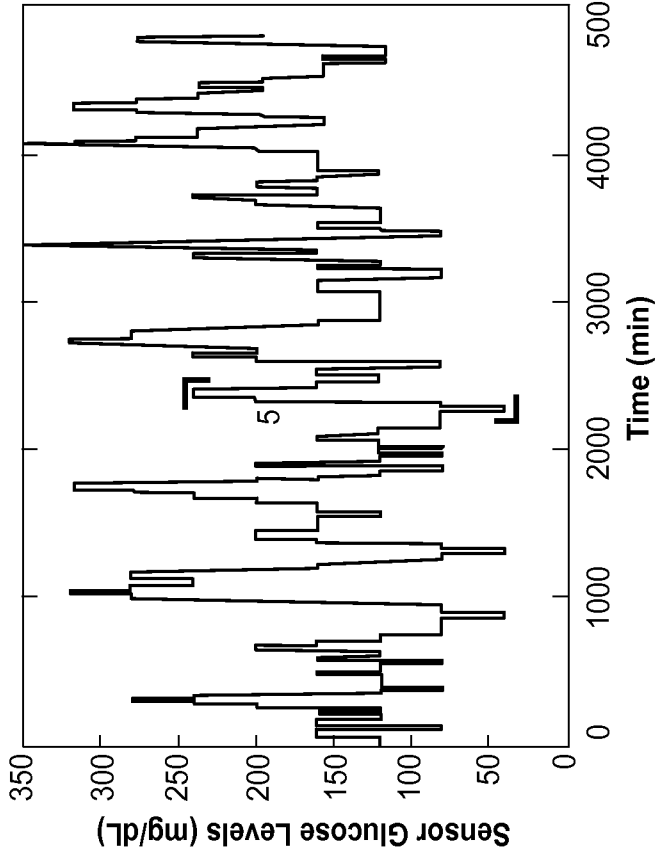


Figure 2C

**Figure 3A****Figure 3B****Figure 3C****Figure 3D**

DySF by Transition Density Profiles

- Continuous glucose data is divided into even bins. (40 mg/dl)
- Monotonic glucose transition magnitudes are established
- Transitions are separated into the timeinterval necessary to complete each change.



DySF is the daily weighted number of large (>1) monotonic glucose transitions magnitudes that occur in less than one hour.

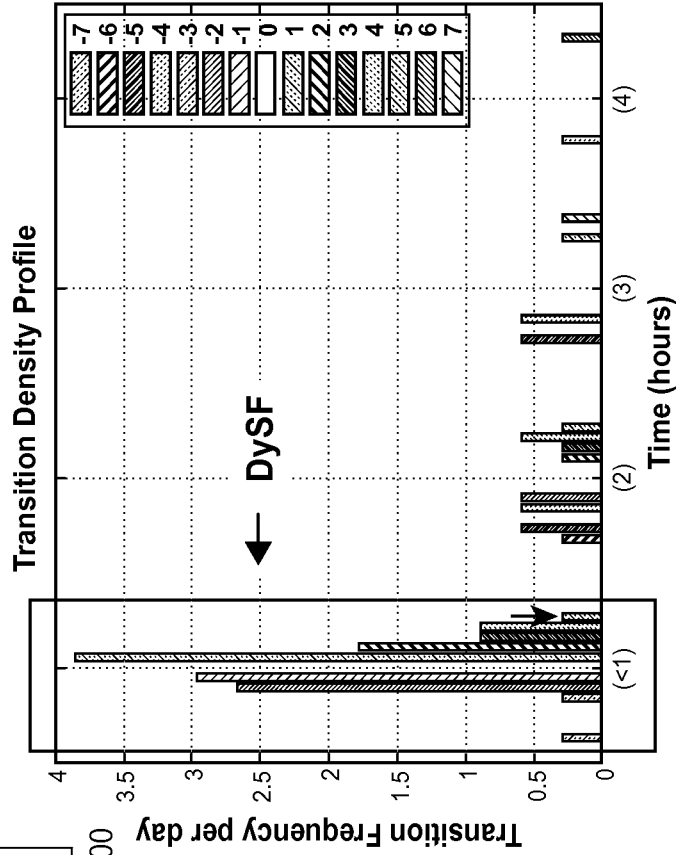


Figure 4

Metric	Patient3	Mean of non-diabetic controls*	Fold Difference
Mean Glucose (mg/dL)	121.9	96.84	1.26
SD	55.2	14.13	3.91
MODD	57.5	14.41	3.99
CONGA(1)	35.7	12.91	2.77
CONGA(2)	54.8	15.89	3.45
CONGA(4)	71.5	18.26	3.92
MAGE	90.1	32.14	2.80
Time hyperglycemic (%)	15.6	0	
Time hypoglycemic (%)	22.0	6.12	3.59
Area under the curve Hyperglycemic (mg*min/dL)	41220		
Area under the curve Hypoglycemic (mg*min/dL)	31315		

Figure 5

Name	Formula	Description
Mean	$\frac{\sum_{t=t_1}^{t_n} BG_t}{n}$	n = number of observations (number of glucose readings for a given individual)
Standard deviation (SD)	$\sqrt{\frac{\sum_{t=t_1}^{t_n} (BG_t - \overline{BG})^2}{n-1}}$	$\overline{BG}$ = mean glucose reading n = number of observations
MODD	$\frac{\sum_{t=t_1}^{t_k} BG_t - BG_{t-1440} }{k}$	k = number of observations where there is an observation at the same time 24 h (1440 min) ago (Molnar GD et. al. 1972)
CONGA(n)	$\sqrt{\frac{\sum_{t=t_1}^{t_k} (D_t - \overline{D})^2}{k-1}}$ where $D_t = BG_t - BG_{t-m}$ $\overline{D} = \frac{\sum_{t=t_1}^{t_k} D_t}{k}$ and $\overline{D}_t = \frac{\sum_{t=t_1}^{t_k} D_t}{k}$	k = number of observations where there is an observation $n \times 60$ min ago $m = n \times 60$ (McDonnell et.al 2005)
MAGE	$\sum \frac{\lambda}{x}$ if $\lambda > v$	λ = the absolute value difference between sequential glucose peaks and nadirs x = number of valid observations $v = 1$ SD of mean glucose (Fritzsche et.al. 2011; Baghurst, 2011; Service FJ et. al. 1970)

Figure 6

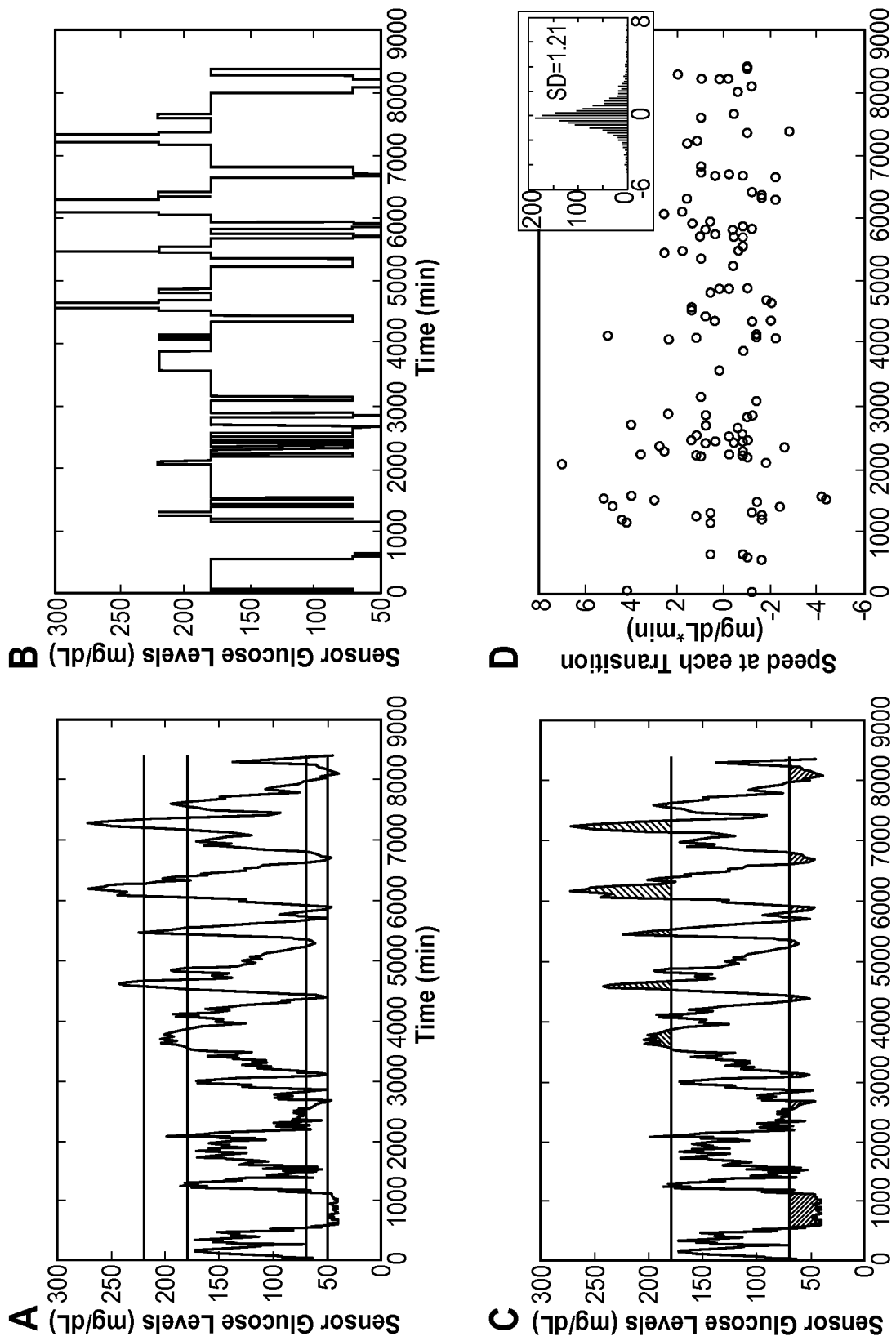


Figure 7

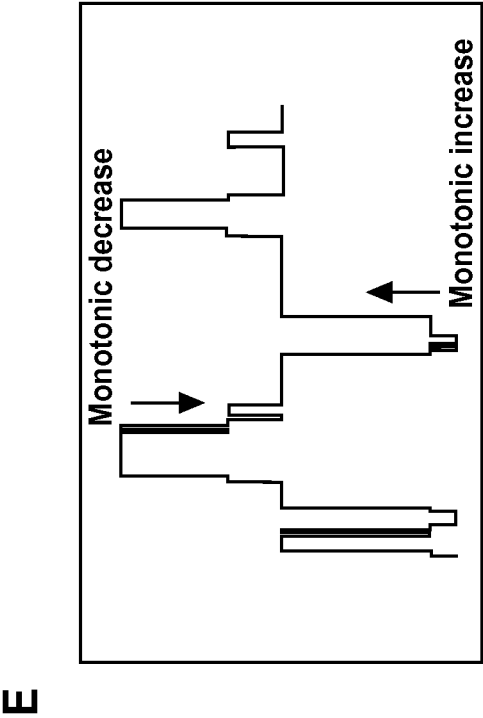
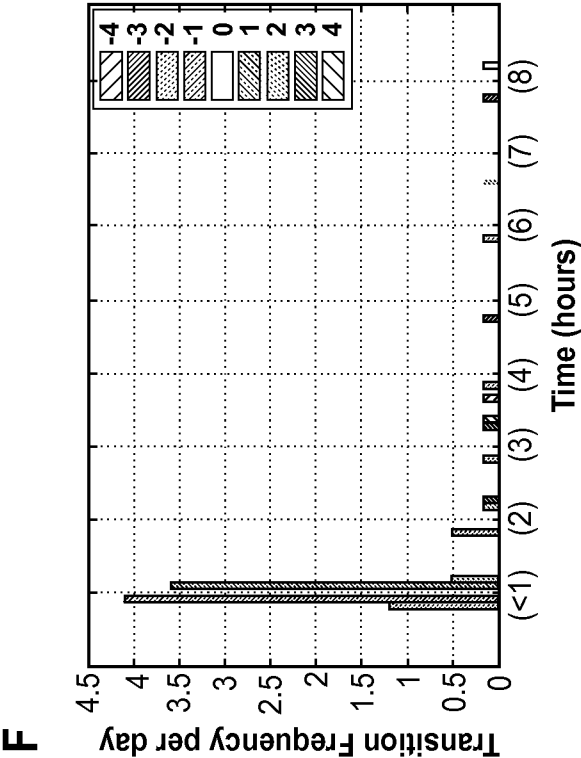


Figure 7

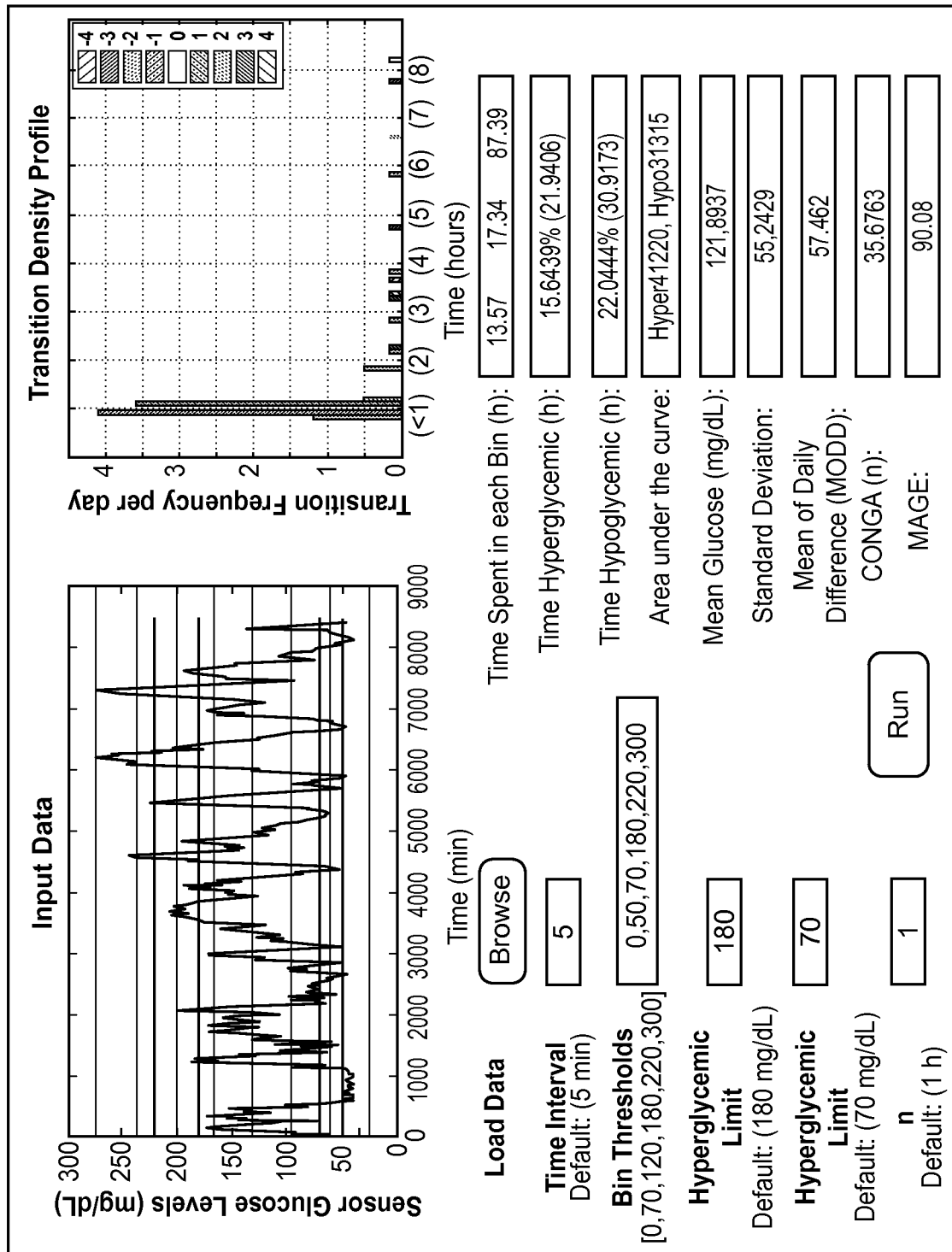
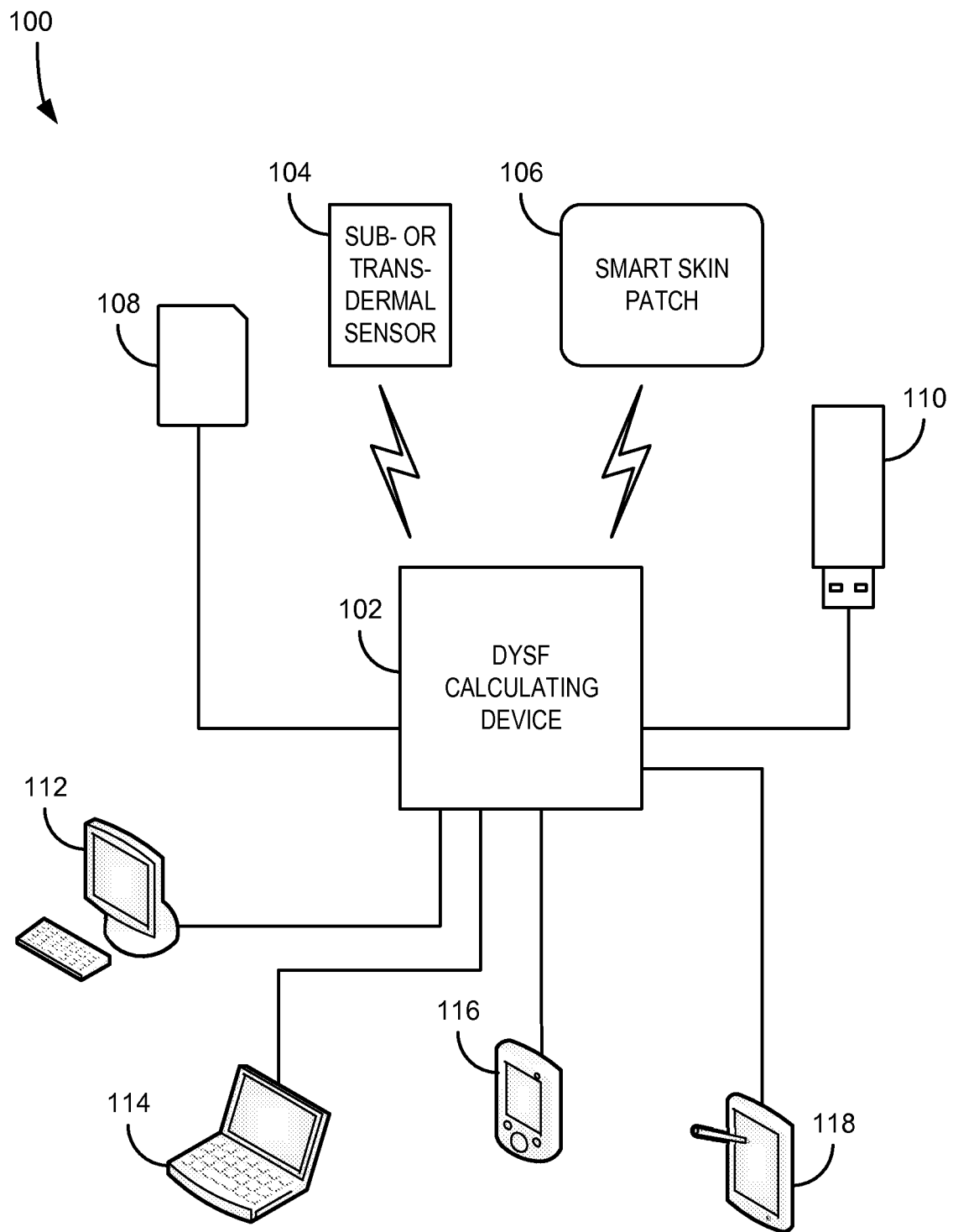
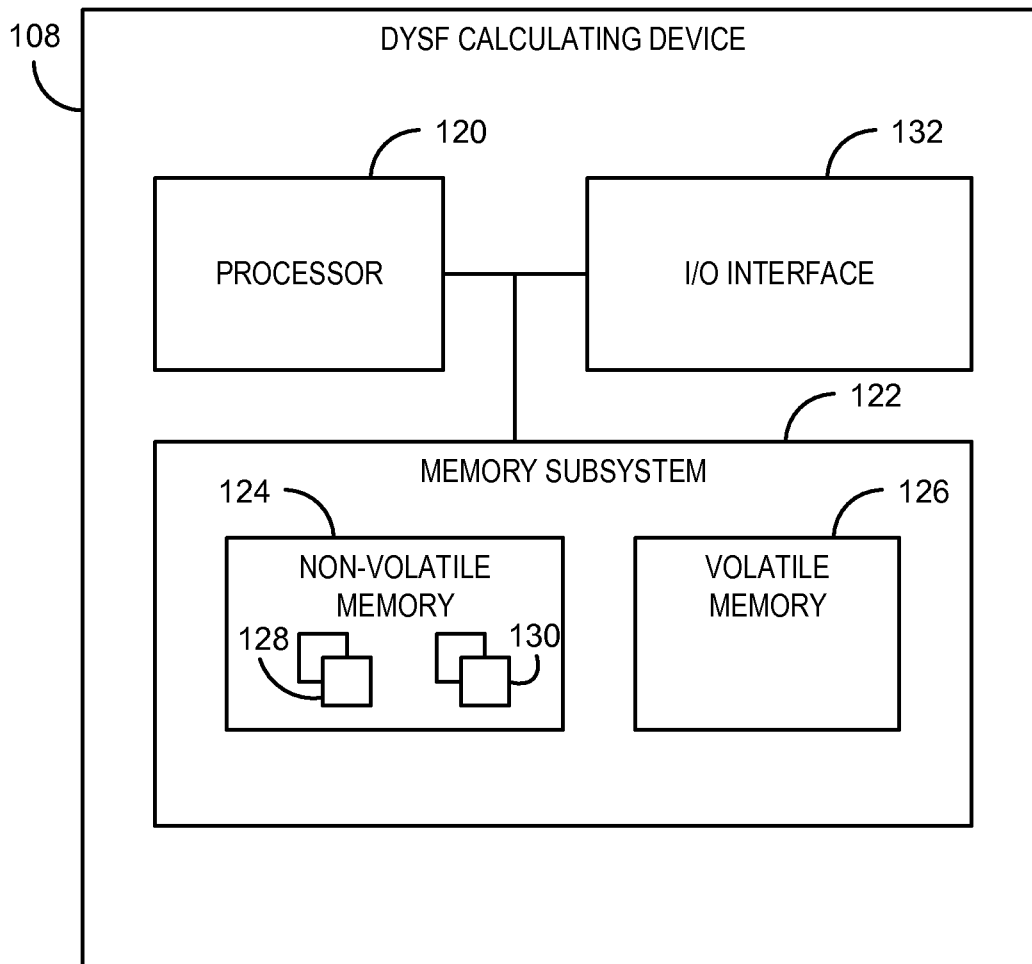
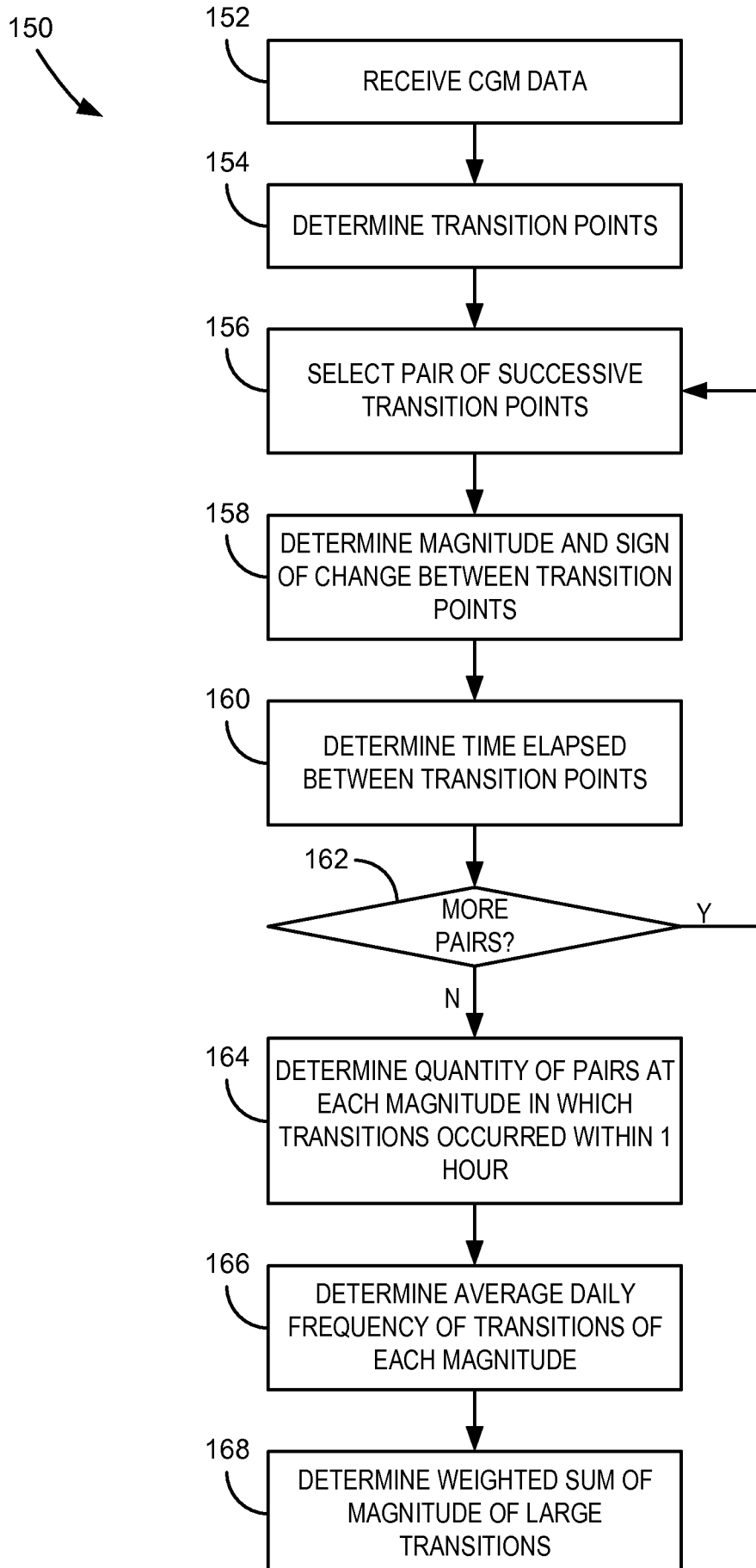


Figure 8

**Figure 9**

**Figure 10**

**Figure 11**

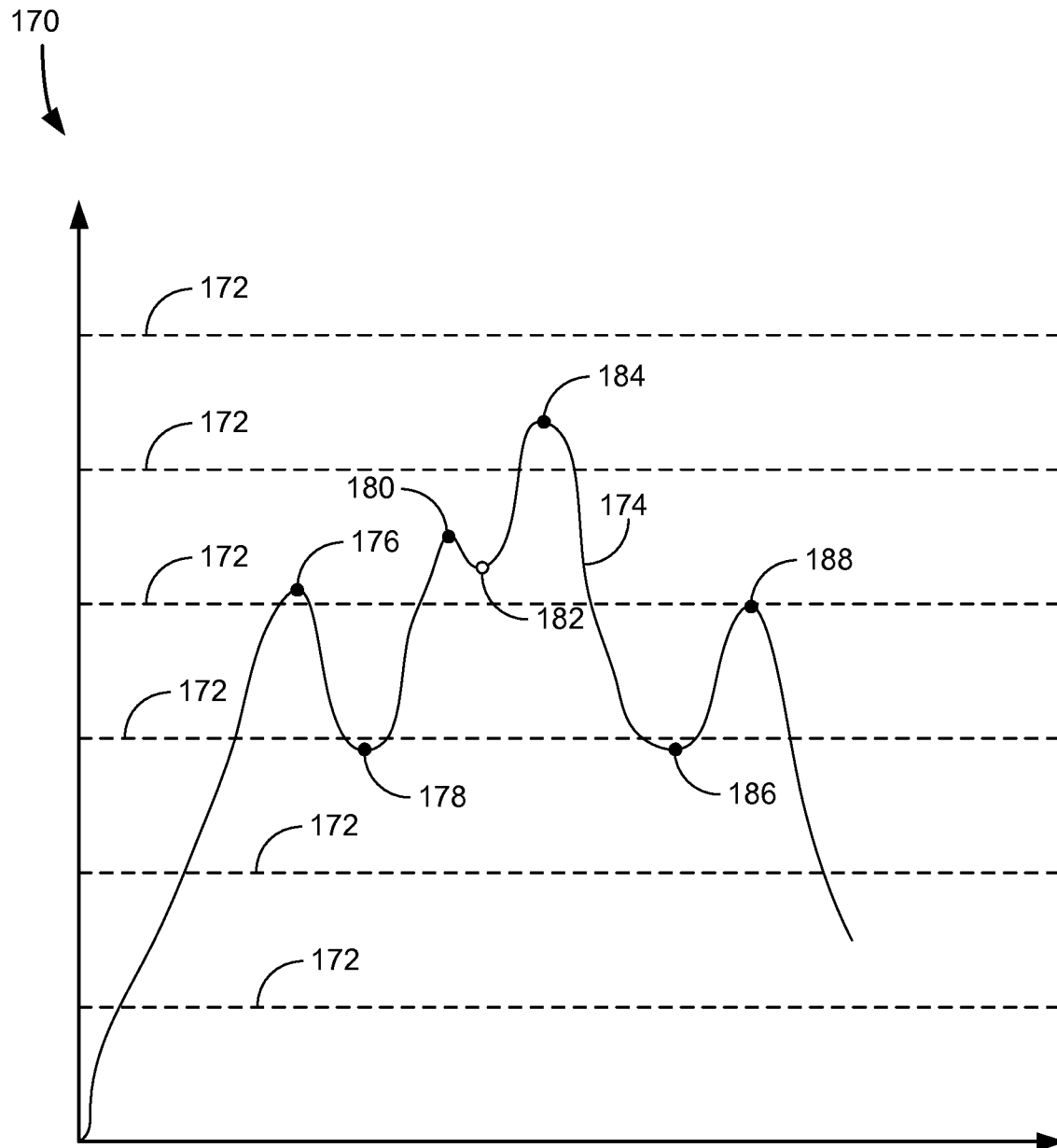
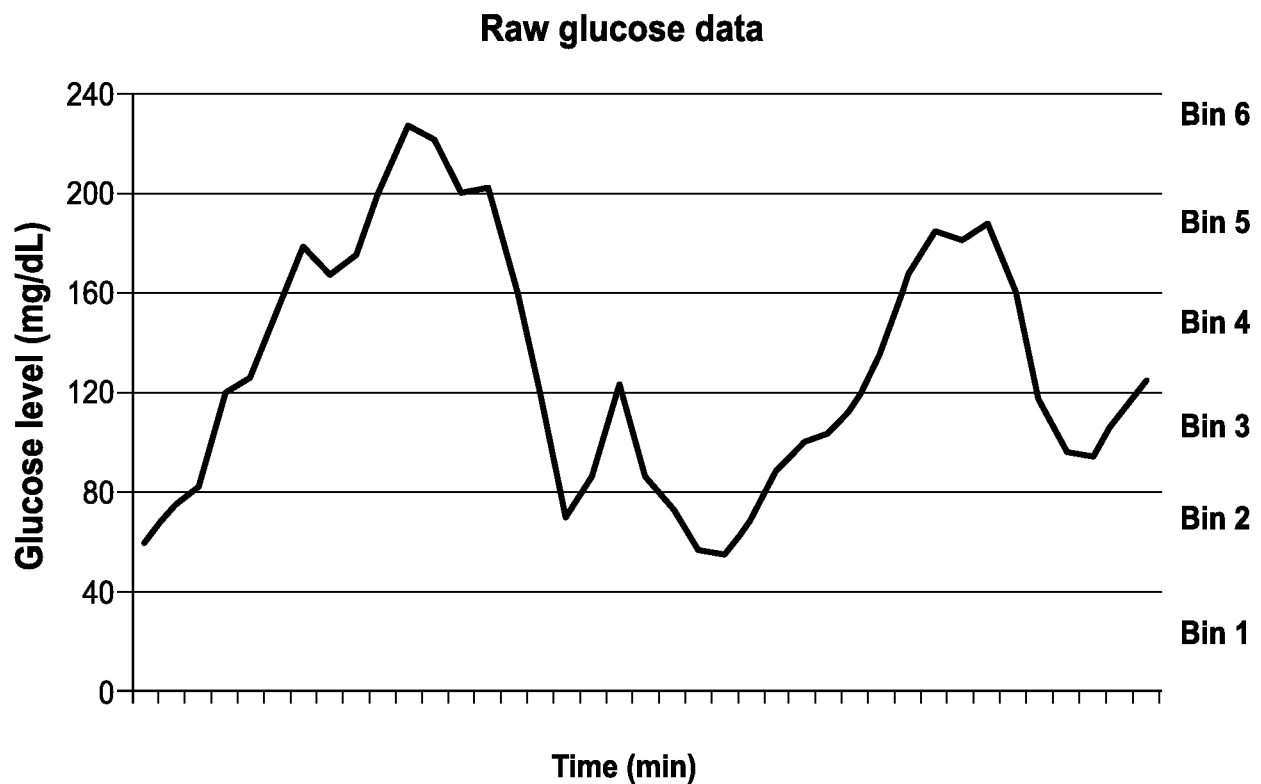


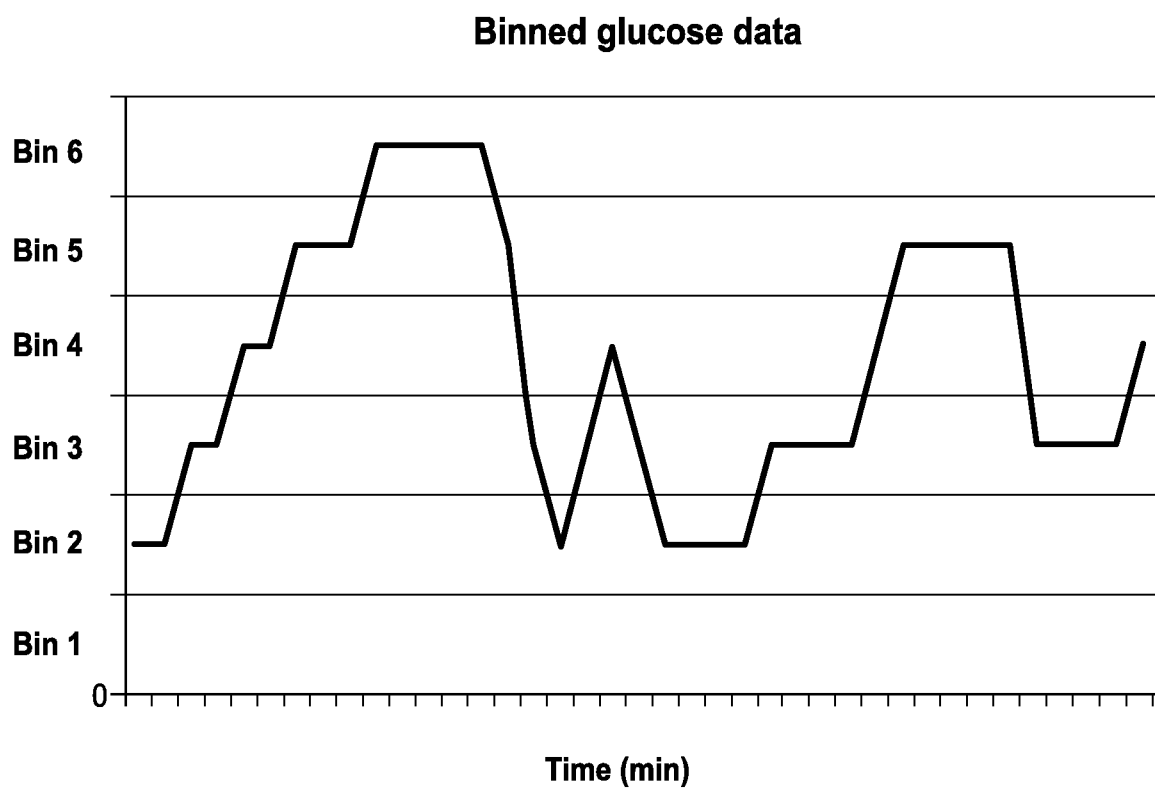
Figure 12

Step 1: Raw Data

The horizontal lines on the graph demark the separate 40 mg/dL Bins
Each tick mark on the X-axis represents 5 min

Figure 13

Step 1B: Bin Data

**Figure 14**

**Step 2: Identify monotonic transitions &
Transition time intervals**

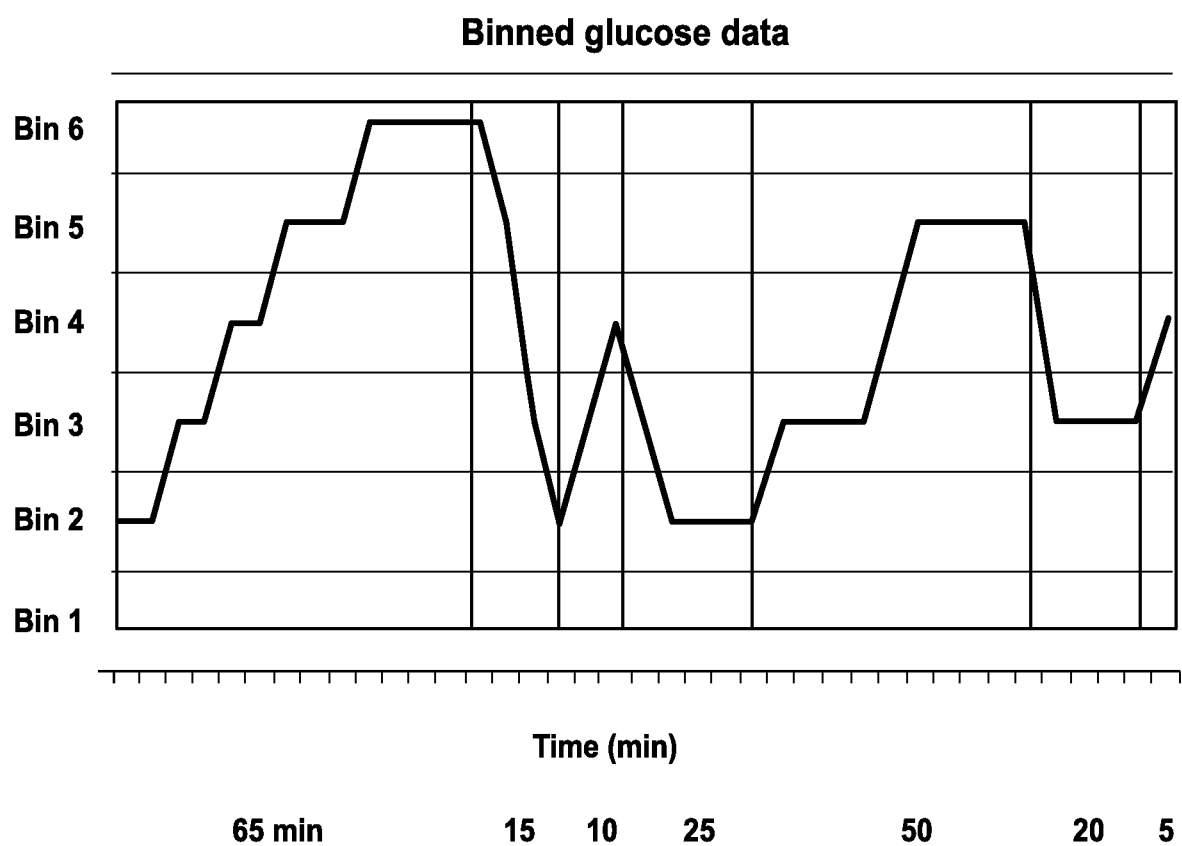
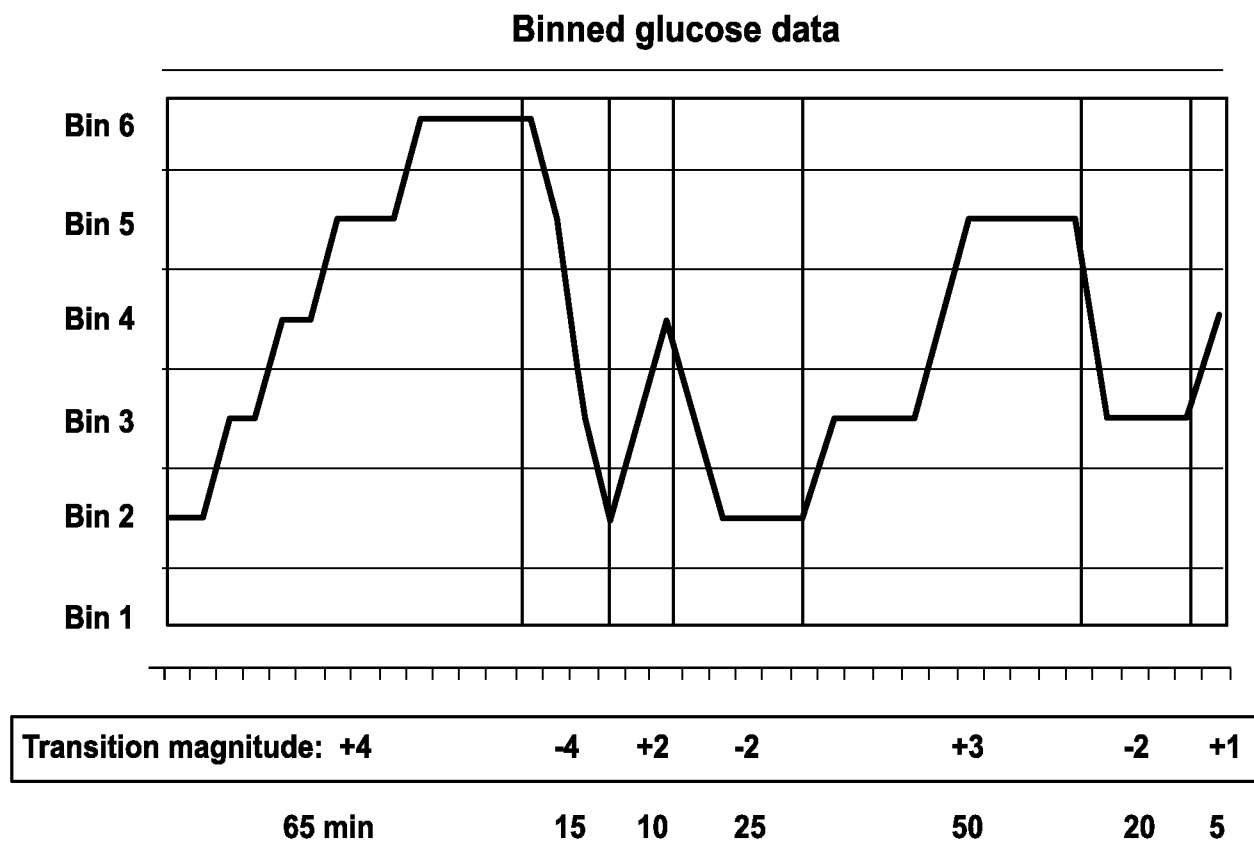
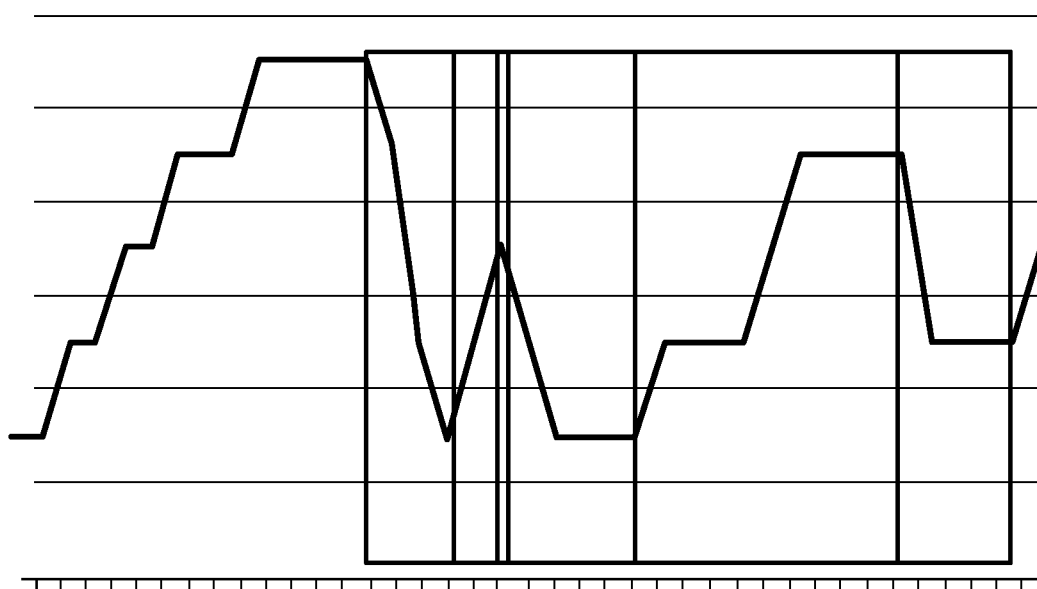


Figure 15

Step 3: Calculate magnitude of monotonic transitions**Figure 16**

Step 4: Add up relevant transitions

Properties of “Relevant transitions”: transition time interval < 1hr and transition magnitude > |1|

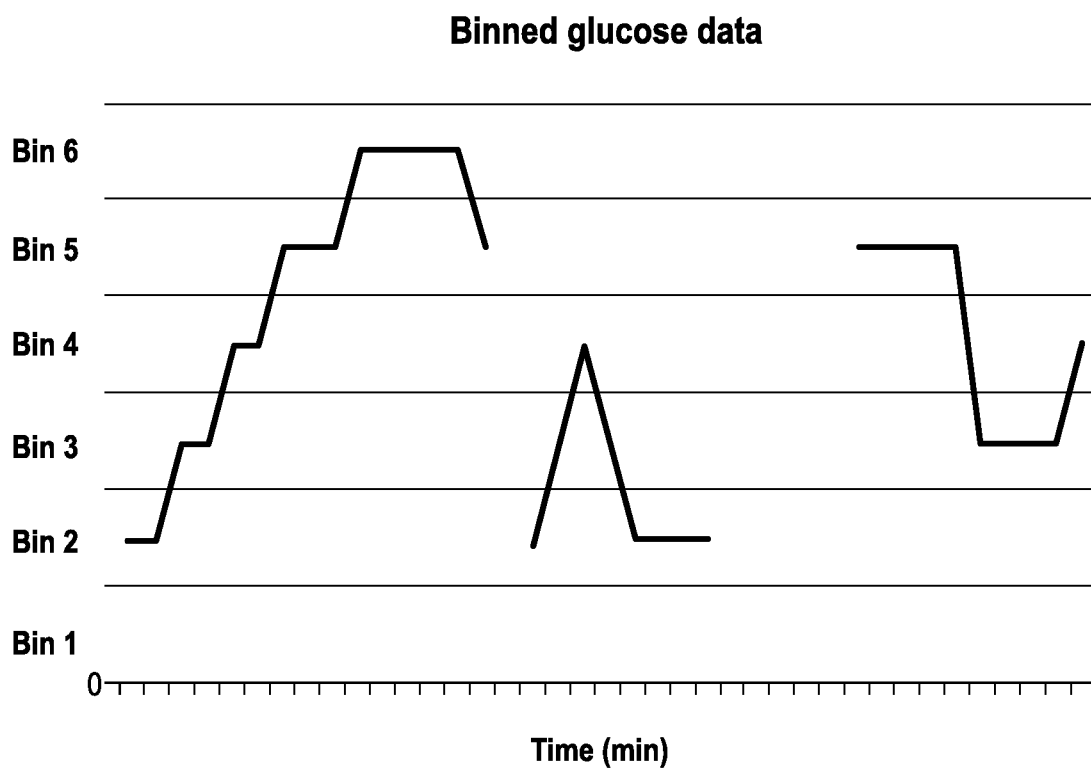


Magnitude of transitions that occur within 1 hour: +4 -4 +2 -2 +3 -2 +1

$$\text{DySF} = (1 * | -4 | + 1 * | +2 | + 2 * | -2 | + 1 * | +3 |) \text{ weighted transitions} / 24 \text{ hours}$$

$$= 0.542 \text{ weighted transitions per day}$$

Figure 17

Appendix: Missing Data

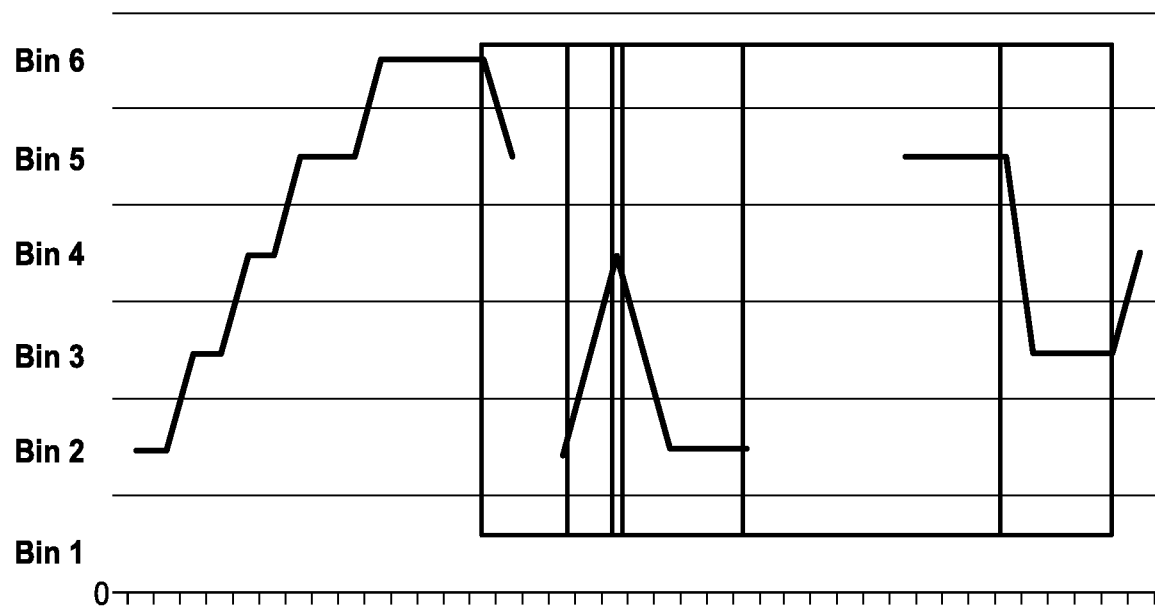
If a transition contains a gap:

If Gap < 15 min, the appropriate time interval is added to the transition

If Gap > 15 min, the transition is considered invalid, and is not used in the calculation of DySF

Figure 18

Appendix: Missing Data

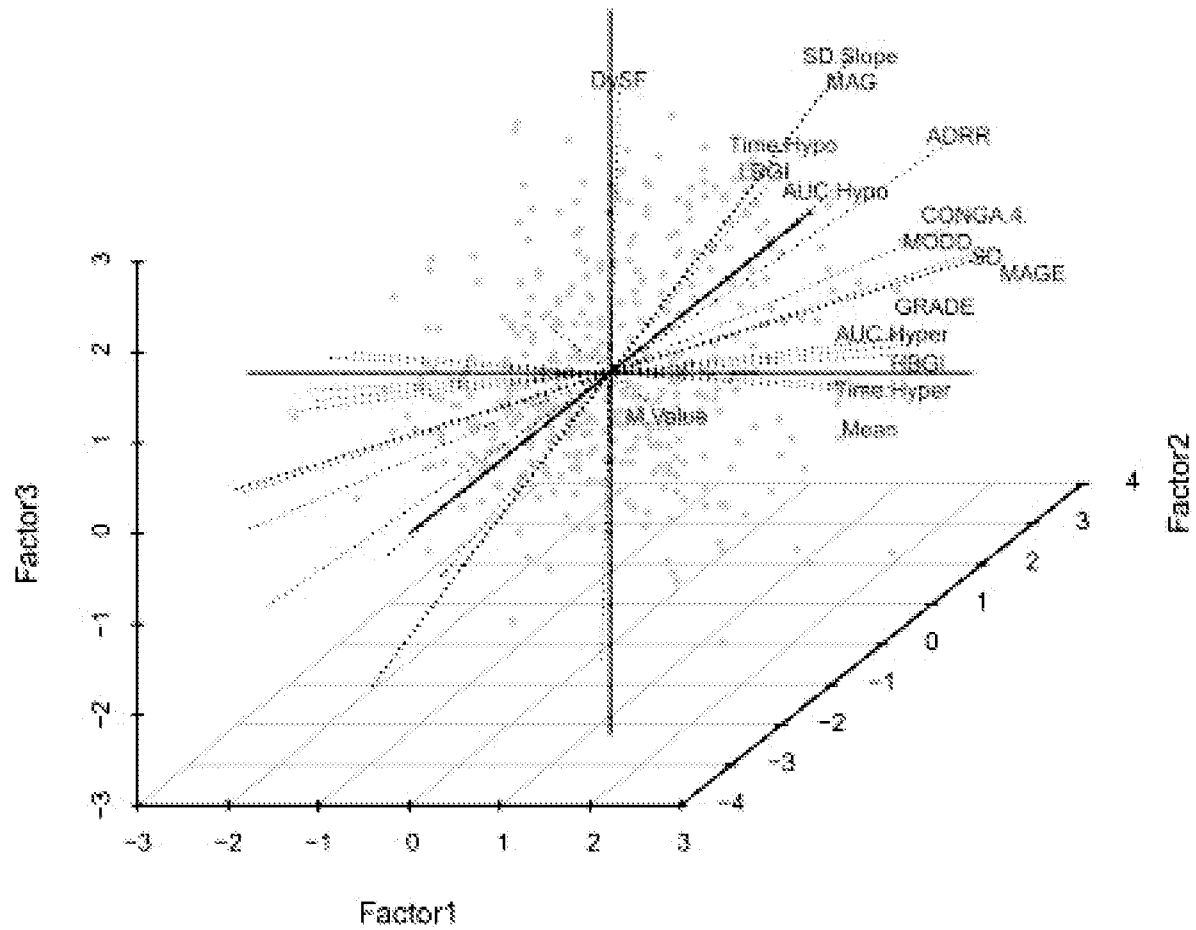


Transitions within 1 hour:	-4	+2	-2	+3	-2
Gap Time:	5 min			25 min	

$$\text{DySF} = (1 \times |-4| + 1 \times |2| + 2 \times |-2| \text{ weighted transitions} / 24 \text{ hours})$$

$$= 0.417 \text{ weighted transitions per day}$$

Figure 19

**Figure 20**

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2012/068831

A. CLASSIFICATION OF SUBJECT MATTER		A61B 5/145 (2006.01) G06F 19/00 (2011.01)
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
A61B 5/145, G06F 19/00		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
PCT Online, USPTO DB, Esp@cenet, DWPI, CIPO (Canada PO), SIPO DB, AIPN, DEPATISnet, VINITI.RU, SCSML.FSSI.RU, PatSearch		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	RENATA A. RAWLINGS, Ph. D. et al. Translating Glucose Variability Metrics into the Clinic via Continuous Glucose Monitoring: A Graphical User Interface for Diabetes Evaluation (CGM-GUIDE). Diabetes technology & therapeutics, Volume 13, Number 12, 2011, pp. 1241-1248	1-45 46-79
Y A	EP 2138944 A2 (ROCHE DIAGNOSTICS GMBH et al.) 30.12.2009, claims, fig. 1-3	1-45 46-79
☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.		
*	Special categories of cited documents:	
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05 March 2013 (05.03.2013)	14 March 2013 (124.03.2013)	
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