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Improved detection of decreased glucose handling capacities via continuous glucose monitoring-derived indices

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Abstract

Background Efficiently assessing glucose handling capacity is a critical public health challenge. This study assessed the utility of relatively easy-to-measure continuous glucose monitoring (CGM)-derived indices in estimating glucose handling capacities calculated from resource-intensive clamp tests.

Methods We conducted a prospective study of 64 individuals without prior diabetes diagnosis. The study performed CGM, oral glucose tolerance tests (OGTT), and hyperglycemic and hyperinsulinemic-euglycemic clamp tests. We validated CGM-derived indices characteristics using an independent dataset from another country and mathematical models with simulated data.

Results A CGM-derived index reflecting the autocorrelation function of glucose levels (AC_Var) is significantly correlated with clamp-derived disposition index (DI), a well-established measure of glucose handling capacity and predictor of diabetes onset. Multivariate and machine learning models indicate AC_Var's contribution to predicting clamp-derived DI independent from other CGM-derived indices. The model using CGM-measured glucose standard deviation and AC_Var outperforms models using commonly used diabetes diagnostic indices, such as fasting blood glucose, HbA1c, and OGTT measures, in predicting clamp-derived DI. Mathematical simulations also demonstrate the association of AC_Var with DI.

Conclusions CGM-derived indices, including AC_Var, serve as valuable tools for predicting glucose handling capacities in populations without prior diabetes diagnosis. We develop a web application that calculates these CGM-derived indices (https://cgm-ac-mean-std.streamlit.app/).

Plain language summary

Diabetes is a chronic disease in which the body cannot effectively use a molecule called insulin or does not produce enough insulin. Insulin is a hormone that regulates a type of sugar called glucose. Early detection of impaired insulin-mediated glucose regulation can be used to predict the onset of diabetes and its complications. This study investigated whether continuous glucose monitors, which are less invasive than those commonly used to diagnose diabetes, could be useful in detecting impaired glucose regulation. Our results suggest that continuous glucose monitoring data could serve as a valuable, less invasive alternative for assessing glucose control in individuals without diagnosed diabetes, allowing for better diagnosis and monitoring of these individuals.

Early detection of declining glycaemic regulatory capacity is crucial for predicting and preventing the onset of diabetes (DM)[1–5]. However, optimal methods for detecting such declines have yet to be established. While glucose tolerance is typically assessed using glycated hemoglobin (HbA1c), fasting blood glucose (FBG) and oral glucose tolerance tests (OGTTs)—which are also used for diagnosing diabetes—these measures provide only snapshot measurements and fail to capture the dynamic nature of glucose regulation under physiological conditions[6–9]. Although hyperinsulinemic-euglycemic and hyperglycemic clamp tests are the gold standards for assessing glucose

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regulatory capacity[10], their resource-intensive nature limits their widespread clinical use.

Continuous glucose monitoring (CGM) has emerged as a powerful tool for capturing glucose dynamics under physiological conditions with ease[11–21]. Recent studies have revealed substantial heterogeneity in glucose fluctuation patterns, even among individuals classified as having normal glucose tolerance (NGT) according to conventional criteria[13,22], suggesting that current diagnostic frameworks may miss subtle, early changes in glucose regulation that precede clinically apparent dysglycaemia. Indeed, decreases in insulin secretion and insulin sensitivity have been reported to begin years before diabetes is diagnosed[3].

Despite the wealth of temporal data provided by CGM, translating these complex time-series patterns into clinically meaningful indicators of glucose regulatory capacity remains a significant challenge. Existing CGM-derived measures, such as the mean amplitude of glycaemic excursions (MAGE) and J-index, have improved our understanding of glucose variability[21]. However, these measures focus primarily on mean or variance-based measures, potentially overlooking critical information embedded in temporal dynamics. Furthermore, the relationship between these CGM-derived measures and established indices of glycaemic regulatory capacity, such as the disposition index (DI, a product of insulin sensitivity and secretion)[2] remains poorly understood, particularly in individuals with NGT.

Here, we propose an analytical framework that uses the autocorrelation function of glucose to quantify the regulatory capacity of glucose homeostasis. In a dataset of 64 individuals with no prior diagnosis of diabetes, this framework identified a previously unrecognized subgroup of individuals who, despite meeting NGT criteria, have reduced DI comparable to those with impaired glucose tolerance (IGT). Moreover, measures of glucose autocorrelation and variability were independently associated with DI.

Methods

Study design and population

This study was conducted in accordance with the Declaration of Helsinki and its amendments, and was approved by the ethics committee of Kobe University Hospital (Approval No. 1834; Kobe, Japan). Written informed consent was obtained from all participants. Study participants who had no previous diagnosis of diabetes and were over 20 years old were recruited from Kobe University Hospital (Hyogo, Japan) from January 2016 to March 2018. Exclusion criteria were: (1) taking medications that affect glucose metabolism (e.g., steroids, β blockers); (2) patients with psychiatric disorders; (3) pregnant or breast-feeding women; and (4) deemed unfit for any other reason by attending physicians.

The study participants initially underwent a 75-g oral glucose tolerance test (OGTT) in the morning after an overnight fast. Following the OGTT, they wore a continuous glucose monitoring (CGM) device (iPro; Medtronic, Minneapolis, MN, USA) for more than 72 h. Within 7 days after the OGTT, the participants underwent a consecutive hyperglycemic and hyperinsulinemic-euglycemic clamp test.

A total of 70 participants were initially enrolled. One participant taking a β blocker, two participants with missing CGM data, two participants with protocol deviation, and one participant with missing OGTT and/or clamp data were excluded from the analysis. Consequently, data from 64 participants were used in the analysis. The sample size of 64 participants closely aligned with that of 57 individuals investigated in a previous study, where statistically significant correlations between CGM-derived indices and the ability to regulate blood glucose were demonstrated[14]. Of note, with a type I error of 0.05, a power of 0.8, and an expected Spearman correlation coefficient of 0.35, a sample size of 66 (Bonett and Wright's method) or 64 (Caruso and Cliff's method) was required to detect a significant difference from zero in the correlation coefficient. This sample size estimation was performed using SPSS version 29 (SPSS Inc.).

OGTT, consecutive hyperglycemic and hyperinsulinemic-euglycemic clamps, and CGM

In a standard 75-g OGTT, venous blood samples were collected at 0, 30, 60, 90, and 120 min after glucose ingestion for measurement of plasma glucose and serum insulin levels (measured by the hexokinase UV method (SEKISUI Medical Co., Ltd., Japan) and chemiluminescent enzyme immunoassay (Minaris Medical Co., Ltd., Japan), respectively). Given the low proinsulin-to-insulin ratios (approximately 0.052 in normal glucose tolerance (NGT) and 0.078 in type 2 diabetes mellitus (T2DM)[23]) and the minimal cross-reactivity of this chemiluminescent enzyme immunoassay (approximately 2%[24]), the potential impact of proinsulin on our insulin measurements and derived indices would be minimal compared to the inter-individual variation observed in our study (e.g., clamp disposition index values of 40.8 in NGT versus 13.9 in T2DM) (Supplementary Table 1). In our study population, which consisted primarily of individuals with no prior diagnosis of diabetes, we did not find any measurements that fell below the detection limit of insulin, which is approximately 0.5 mU/L.

The hyperglycemic and hyperinsulinemic-euglycemic clamp analysis was performed with the use of an artificial endocrine pancreas (STG-55; Nikkiso Co., Ltd, Tokyo, Japan) as described previously[25]. In brief, from 0 to 90 min, a hyperglycemic clamp was performed by intravenous infusion of a bolus of glucose (9622 mg/m^2) within 15 min followed by that of a variable amount of glucose to maintain the plasma glucose level at 200 mg/dL. Ten minutes after the end of the hyperglycemic clamp, a 120-min hyperinsulinemic-euglycemic clamp was initiated by intravenous infusion of human regular insulin (Humulin R, Eli Lilly Japan K.K., Kobe, Japan) at a rate of 40 mU m^{-2} min^{-1} and the hyperinsulinemic state was maintained to achieve a target glucose level of the fasting level and a serum insulin concentration of 100 μU/ml. Plasma glucose concentrations were measured every minute during the clamp and averaged over a 5-min period. The data on plasma glucose and serum insulin were also collected before and at 5, 10, 15, 60, 75, 90, 100, 190, and 220 min after the onset of the clamp tests.

Data from the CGM were used in the analysis for the 72-h period from the next days fitted with iPro. Capillary blood glucose levels were measured at least three times per day using a glucometer (Accucheck Performa, Roche Diabetes Care Japan K.K., Tokyo, Japan), which was required for calibration of the CGM system. CGM was performed for an average of 5.5 days (SD 0.7) for all participants. The CGM measurements were completed before the clamp tests for all participants.

Study design and population of a previously reported dataset

We also performed an analysis using a publicly available dataset of CGM (Dexcom G4 CGM System; Dexcom, Fort Lauderdale, FL, USA), OGTT, and steady-state plasma glucose (SSPG) test outcomes from a previously reported study[14]. SSPG indicates insulin sensitivity, and was measured by infusing octreotide, insulin, and glucose, as previously described[14]. The participants of that study, recruited from the San Francisco Bay Area, had no previous diagnosis of diabetes[14]. Among the study participants (32 females and 25 males), 5, 14, and 38 individuals met their criteria of "type 2 diabetes" (HbA1c ≥ 6.5%, FBG ≥ 126 mg/dL, or 2-h glucose during 75-g OGTT ≥ 200 mg/dL), "pre-diabetes" (HbA1c > 5.7% and <6.5%, FBG 100–125 mg/dL, or 2-h glucose during 75-g OGTT 140–199 mg/dL) and "normoglycemia" (glucose-related parameters below the diagnostic thresholds for pre-diabetes)[14]. The study was approved by the Stanford Internal Review Board (IRB 37141), and written consent was obtained for all participants.

CGM-derived parameters

CGM_Mean and CGM_Std represent the mean and standard deviation of glucose values measured by CGM, respectively. CONGA, LI, JINDEX, HBGI, GRADE, MODD, MAGE, ADRR, MVALUE, and MAG were calculated using EasyGV software[21]. Here, we introduced indices, the mean (AC_Mean) and the variance (AC_Var) of the autocorrelation function of glucose values at lags 1–30 with a lag of 5 min. The code that calculates AC_Mean and AC_Var is available from the repository (https://github.

com/HikaruSugimoto/CGM_AC)[26] and the web application (https://cgm-ac-mean-std.streamlit.app/). While autocorrelation function has been used to analyze this type of time series data[27,28], it has not been thoroughly investigated to date. AC_Mean and AC_Var used in analyzing blood glucose levels after consuming standardized meals[14] were calculated from the autocorrelation function at lags 1–10, as we had CGM data available for only 2.5 h after standardized meals were consumed.

DTW_Low, DTW_Mod, and DTW_Sev are previously proposed CGM-derived indices that represent the dysregulation of glycemia[14]. These indices were calculated by a previously reported method[14]. In brief, the time series data of CGM were fragmented into sliding windows of 2.5 h, with a 75% overlap. Then, by applying spectral clustering, three clusters of glucose patterns (low, moderate, and severe) were identified, and the fraction of time in each category was defined as DTW_Low, DTW_Mod, and DTW_Sev, respectively.

Calculation of clinical indices

As previously described[29,30], we calculated indices related to glucose handling capacities, as follows.

Insulinogenic index (I.I):

Ratio of the increment of immunoreactive insulin (IRI) to that of plasma glucose at 30 min after the onset of the OGTT.

Composite index:

$$\left[10000/\sqrt{\mathrm{FPG}\times\mathrm{FIRI}\times\mathrm{G}\times\mathrm{I}}\right],$$

where FPG, FIRI, G, and I are fasting plasma glucose, fasting IRI, mean blood glucose levels, and mean serum IRI concentrations during the OGTT, respectively.

Oral DI:

Product of the composite index and the ratio of the area under the insulin concentration curve from 0 to 120 min to that for plasma glucose from 0 to 120 min, without using the data measured at 90 min, in the OGTT.

AUC_IRI:

Incremental area under the IRI concentration curve from 0 to 10 min during the hyperglycemic clamp.

Insulin sensitivity index (ISI):

The mean glucose infusion rate during the final 30 min of the clamp (mg/kg/min) divided by both the plasma glucose (mg/dL) and serum insulin (μU/mL) levels at the end of the clamp and then multiplying the resulting value by 100.

Clamp DI:

The product of AUC_IRI and ISI.

Metabolic clearance rate of insulin (MCRI):

Ratio of insulin infusion rate to the steady-state plasma insulin concentration during the hyperinsulinemic-euglycemic clamp test.

Mathematical model estimating insulin sensitivity, secretion, and clearance

To estimate insulin sensitivity, insulin secretion, and insulin clearance from clamp tests, we constructed a mathematical model of the feedback loop that links glucose and insulin as shown in a previous study[29] as follows:

$$\begin{aligned}\frac{dG}{dt} &= \mathrm{flux1}-\mathrm{flux2}+\mathrm{flux3}-\mathrm{flux4}+\mathrm{influx}\,G\\ &= k_1Y-k_2G+\frac{k_3}{k_8+I}-k_4GI+f_1(t)\end{aligned} \tag{1}$$

$$\frac{dI}{dt} = \mathrm{flux6}-\mathrm{flux7}+\mathrm{influx}I = k_6X-k_7I+f_2(t) \tag{2}$$

$$\frac{dY}{dt} = -\mathrm{flux1}+\mathrm{flux2} = -k_1Y+k_2G \tag{3}$$

$$\frac{dX}{dt} = \mathrm{flux5}-\mathrm{flux6} = k_5Y-k_6X, \tag{4}$$

where the variables G and I denote blood glucose and insulin concentrations, respectively. The variable Y denotes the effective glucose on induction of variable X, which can be regarded as secreted insulin from β-cells. The fluxes, influx G and influx I denote glucose and insulin infusions, respectively. These fluxes were estimated using the previously reported method[29], as follows.

For each of the 64 participants, the parameters of the model to reproduce the time course were estimated by a meta-evolutionary programming method to search the minimum globally, followed by application of the nonlinear least squares technique to search the minimum locally, as previously described[30]. Each parameter of the model for serum glucose and insulin concentration was estimated in the range from 10^{-4} to 10^{4}. For these methods, the parameters were estimated to minimize the objective function value, which is defined as residual sum of the square (RSS) between the actual time course obtained by clamp analyses and the model trajectories. RSS used in the model for serum glucose and insulin concentration was given by the following equation:

$$\begin{aligned}\mathrm{RSS} = &\frac{n_I}{n_G+n_I}\sum_{i=1}^{n_G}\left[G(t_i)-G_{\mathrm{sim}}(t_i)\right]^2\\ &+\frac{n_G}{n_G+n_I}\sum_{i=1}^{n_I}\left[I(t_i)-I_{\mathrm{sim}}(t_i)\right]^2,\end{aligned} \tag{5}$$

where n_G and n_I are the total numbers of time points of measuring blood glucose and insulin, respectively, and t_i is the time of i-th time point. $G(t)$ is the time-averaged blood glucose concentration within the time range $(t-5)$ min to t min with every 1-min interval, $I(t)$ is the blood insulin concentration at t min. $G_{\mathrm{sim}}(t)$ and $I_{\mathrm{sim}}(t)$ are simulated blood glucose and insulin concentrations, respectively. Blood glucose and insulin concentrations of each subject were normalized by dividing them by the respective maximum value. The numbers of parents and generations in the meta-evolutionary programming were 400 and 4000, respectively. Of note, the previous study showed that this mathematical model was reasonably able to capture the essential characteristics of the time-series data, and k_4, k_5, and k_7 well represent insulin sensitivity, secretion, and clearance, respectively[29]. This simulation was conducted using MATLAB R2021a (https://jp.mathworks.com/).

Mathematical model used for simulating the characteristics of AC_Mean and AC_Var

In simulating the characteristics of AC_Mean and AC_Var, we used a simple and stable model[31], which can be written as follows:

$$\frac{dG}{dt} = -k_{\mathrm{glu}}G-k_{\mathrm{sen}}IG+k_{\mathrm{pro}}+f \tag{6}$$

$$\frac{dI}{dt} = \frac{k_{\mathrm{sec}}}{k_{\mathrm{tim}}}\int_{t-k_{\mathrm{tim}}}^{t}G\,ds-k_{\mathrm{cle}}I \tag{7}$$

where the variables G and I denote blood glucose and insulin concentrations, respectively. Parameter values reported as the averages for healthy subjects were as follows[31]:

$$k_{\mathrm{glu}} = 0.0226,\ k_{\mathrm{sen}} = 5.64\times10^{-5},\ k_{\mathrm{pro}} = 1.93,$$

$$k_{\mathrm{sec}} = 0.074,\ k_{\mathrm{tim}} = 14.9,\ k_{\mathrm{cle}} = 0.1262.$$

We simulated how 24-h profiles of G changed as $k_{\mathrm{sec}}k_{\mathrm{sen}}$ and k_{cle}, which correspond to the DI and insulin clearance, respectively, were changed from one-half to twice as large as the values. Five mg/dL/min glucose was applied for 10 min at 6-, 12-, and 18-h as the external input

of glucose f. We also validated the results using another mathematical model representing both a single meal and daily life[32]. We also calculated the AC_Mean and AC_Var from G added with zero-mean gaussian white noise with variances of 0.25, 0.5, or 1. This simulation was conducted using SciPy v1.10.1[33].

Statistics and reproducibility

We investigated the predictive performance of CGM-derived indices for assessing the glucose handling capacities across five major methodologies: multiple linear regression, partial least squares (PLS) regression, least absolute shrinkage operator (Lasso) regression, random forests, and logistic regression. These regression models were used to estimate the important features for the predictions[34–36]. Of note, these prediction models were conducted as post hoc analyses, not to discuss the sufficiency of the input variables, but to estimate which of the input variables examined in this study are important in the predictions. Given this purpose, we performed only these five models. The input variables for these models consisted of 27 variables: body mass index (BMI), abdomen circumference (Acir), body fat percentage, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), triglycerides (TGs), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), FBG, HbA1c, CGM_Mean, CGM_Std, CONGA, LI, JINDEX, HBGI, GRADE, MODD, MAGE, ADRR, MVALUE, MAG, DTW_Mod, DTW_Sev, AC_Mean, and AC_Var. This modeling was conducted using scikit-learn v1.0.2., a python-based tool kit (https://scikit-learn.org/stable/).

The predictive performance assessment of multiple linear regression included measures such as the coefficient of determination (R^2), the adjusted coefficient of determination (AdjR^2), and Akaike information criterion (AIC). The multicollinearity of the input variables was estimated by the variance inflation factor (VIF). PLS regression was conducted to estimate the importance of the input variables in predicting the DI. The variable importance in projection (VIP) scores[37], which were generated from PLS regression, were used for estimating the importance of the input variables. Lasso regression is a kind of linear regression with L1 regularization[38,39]. The optimal regularization coefficients, lambda, were based on leave-one-out cross validation. For multiple linear regression, PLS regression, Lasso regression, and logistic regression, z-score normalization was performed on each input variable.

Random forest is an ensemble learning method, which generates classification decision trees by selecting subsets of input predictor variables randomly[40]. Random forests have been used to predict T2DM and its complications, and to estimate risk factors associated with T2DM[36,41,42]. The study employed 300 decision trees with Gini as the criterion for determining the best splits. The predictive performance of random forests was assessed using accuracy and F1 score based on leave-one-out cross-validation. The importance of the input variables in predicting glycemic anomaly is based on the permutation and the feature importance function of the random forest function. Boruta[43] was also used to test whether the input variables is usable for the prediction.

Associations between indices were assessed using Spearman's correlation test, and correlation coefficients were reported with 95% confidence intervals (CIs) through bootstrap resampling. The number of resamples performed to form the bootstrap distribution was set at 10000. $P < 0.05$ was considered statistically significant. Benjamini-Hochberg's multiple comparison test was also performed with a significance threshold of $Q < 0.05$.

Hierarchical clustering analysis was also conducted using a method that combines Euclidean distance measure and Ward linkage. It was adopted after Z score normalization. Comparisons among individuals in each cluster were performed by analysis of variance followed by Tukey's honestly significant difference test.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Results

Characterization of glucose dynamics using autocorrelation functions

To more accurately and comprehensively evaluate glucose handling capacity, we developed CGM-derived measures based on autocorrelation functions: AC_Mean and AC_Var (Fig. 1a, Methods). These quantify how quickly glucose autocorrelation decays with increasing time lag, reflecting the degree of similarity between current glucose levels and those several minutes earlier. For example, individuals with higher clamp test-derived disposition index (clamp DI) showed gradual glucose fluctuations characterized by slow autocorrelation decay (e.g., participant #13: clamp DI = 65.7, AC_Mean = 0.62, AC_Var = 0.049), whereas those with lower clamp DI showed rapid fluctuations with steep autocorrelation decay (e.g., participant #46: clamp DI = 11.5, AC_Mean = 0.24, AC_Var = 0.12). Conventional measures failed to capture these differences, as evidenced by participant #46 having a lower mean glucose (95 mg/dL) and standard deviation (16 mg/dL) compared to participant #13 (107 mg/dL and 18 mg/dL, respectively). Based on these observations and the mathematical properties of the autocorrelation function, which remains invariant under standardization (mean = 0, variance = 1), we hypothesized that these measures may encode information about glucose regulatory capacity not captured by conventional measures.

Association between glucose dynamics autocorrelation and disposition index

To test this hypothesis, we examined the relationship between these measures (AC_Mean and AC_Var) and established measures of glucose regulatory capacity, including the disposition index and insulin clearance, which have been reported to predict the development of future T2DM beyond FBG and plasma glucose levels at 120 min during the OGTT (PG120)[1,2]. Participant characteristics are detailed in Methods and Supplementary Table 1.

AC_Mean showed a significant correlation with insulin clearance (k_7, $r = 0.28$; 95% CI: 0.04 to 0.50). AC_Var showed significant correlations with oral DI ($r = -0.28$; 95% CI: −0.51 to −0.02), clamp DI ($r = -0.31$; 95% CI: −0.52 to −0.07), insulin sensitivity (k_4, $r = -0.31$; 95% CI: −0.52 to −0.06) and insulin clearance (k_7, $r = -0.31$; 95% CI: −0.54 to −0.06) (Fig. 1b). These correlations remained significant after Benjamini–Hochberg multiple testing ($Q < 0.05$). AC_Mean also showed a statistically significant correlation with k_4k_5 (corresponding to the clamp DI, $r = 0.29$; 95% CI: 0.05 to 0.50). AC_Var showed statistically significant correlations with k_4k_5 ($r = -0.29$; 95% CI: −0.50 to −0.05) and metabolic clearance rate of insulin (MCRI, $r = -0.33$; 95% CI: −0.55 to −0.08). Furthermore, AC_Mean and AC_Var calculated from different lags were significantly correlated with k_7, oral DI, and clamp DI (Supplementary Fig. 1a).

For comparison, we assessed conventional measures including FBG, HbA1c, and CGM-derived measures such as mean (CGM_Mean), standard deviation (CGM_Std), and dynamic time warping-based measures (DTW_Low, DTW_Mod, and DTW_Sev). The DTW-based measures have been shown to identify individuals with decreased ability to control blood glucose in populations primarily classified as having NGT[14]. These measures were not significantly correlated with all of oral DI, clamp DI, k_4, and k_7, with which AC_Var was significantly correlated, but were significantly correlated with PG120, insulinogenic index (I.I.), and the composite index, with which AC_Var was not significantly correlated (Fig. 1b), suggesting that autocorrelation-based measures capture different aspects of glucose regulation than conventional measures.

Combined use of CGM-derived indices improves prediction of glucose handling capacity

The dynamic time warping used to calculate DTW_Low, DTW_Mod and DTW_Sev globally aligns time series data and may not fully capture autocorrelated structural information[44]. As a result, these DTW-based measures primarily reflect the mean and variance of glucose levels[45,46]. In contrast, AC_Var, calculated from the autocorrelation function of glucose levels, can

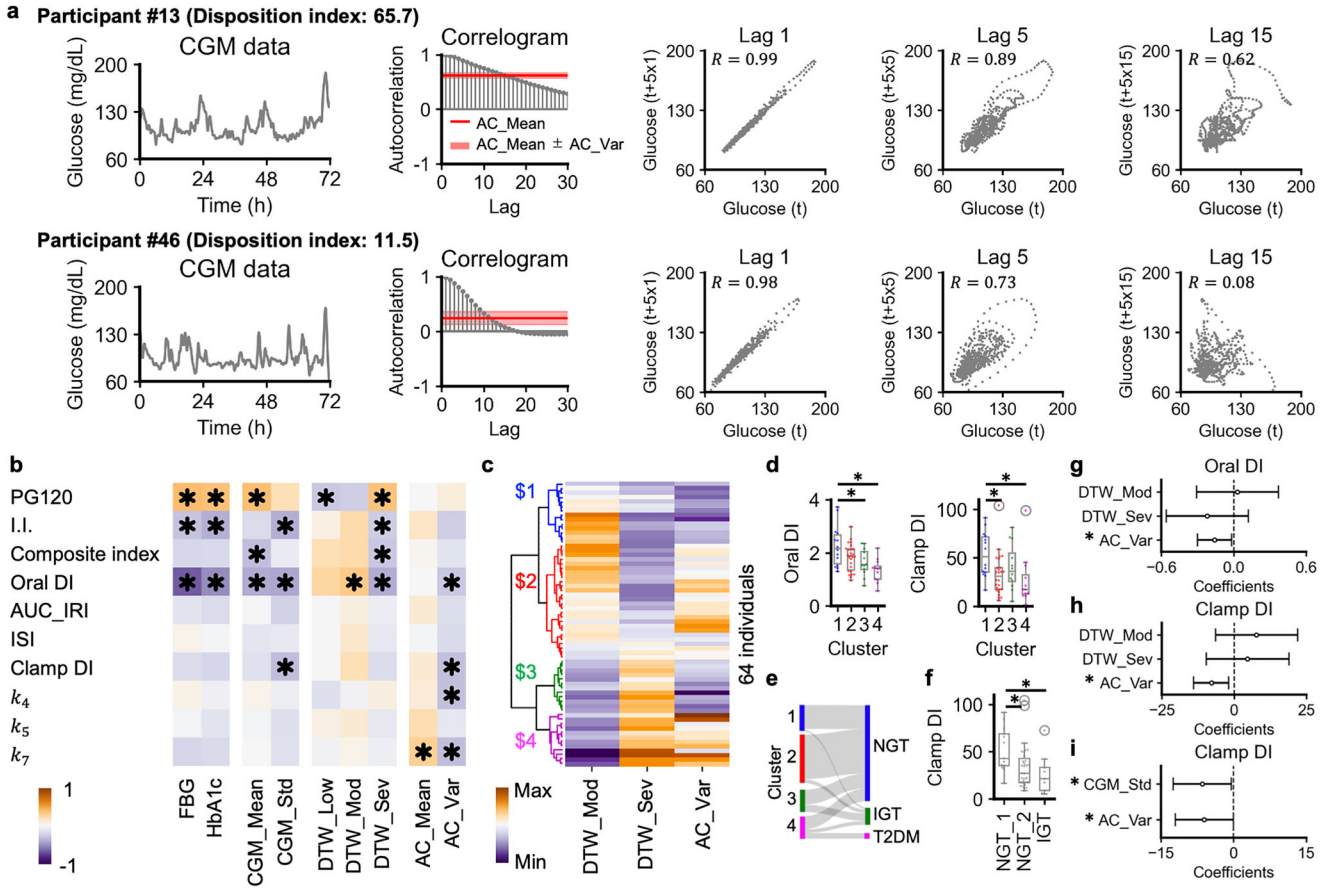

Fig. 1 | Characterization of glucose dynamics using autocorrelation functions. **a** Representative continuous glucose monitoring (CGM) time series data and their corresponding autocorrelation functions from two participants. Red lines indicate the mean autocorrelation values (AC_Mean), with red shading indicating the variance (AC_Var). The autocorrelation was calculated with different time lags, where lag 1 represents the correlation (*R*) between glucose measurements taken 5 min apart (Glucose (t) vs Glucose (t + 5 × 1)), lag 5 represents 25-min intervals (Glucose (t) vs Glucose (t + 5×5)) and lag 15 represents measurements 75-min intervals (Glucose (t) vs Glucose (t + 5 × 15)). **b** Heatmap of Spearman's correlation coefficient with *P* values for testing the hypothesis of no correlation. The analysis is based on data from 64 participants. **c** Hierarchical clustering of CGM-derived indices (DTW_Mod, DTW_Sev, and AC_Var) in 64 participants using Euclidean distance as a metric with the Ward method. Rows represent individual participants and columns show the standardized values of the CGM-derived indices. **d** Box plots of oral DI and clamp DI for each cluster. Each point corresponds to the value for a single participant. *$P < 0.05$. The *P* values corresponding to the symbols are as follows: Cluster 1 (Oral DI) vs Cluster 3 (Oral DI), 0.038; Cluster 1 (Oral DI) vs Cluster 4 (Oral DI), 0.006; Cluster 1 (Clamp DI) vs Cluster 2 (Clamp DI), 0.034; Cluster 1 (Clamp DI) vs Cluster 4 (Clamp DI), 0.021. **e** Sankey diagram showing the relationship between cluster assignment and diabetes diagnosis. **f** Clamp DI values stratified by glycaemic subtypes: NGT_1 (NGT in cluster 1), NGT_2 (NGT in cluster 2 or 4), and IGT. The *P* values corresponding to the symbols are as follows: NGT_1 (Clamp DI) vs NGT_2 (Clamp DI), 0.047; NGT_1 (Clamp DI) vs IGT (Clamp DI), 0.030. **g**–**i** 95% confidence intervals for regression coefficients showing the contributions of: (**g**) DTW_Mod, DTW_Sev and AC_Var to oral DI; (**h**), DTW_Mod, DTW_Sev and AC_Var to clamp DI; and (**i**), CGM_Std and AC_Var to clamp DI. PG120, plasma glucose concentration at 120 min during the oral glucose tolerance test; I.I., insulinogenic index; oral DI, oral disposition index; AUC_IRI, area under insulin curve during the first 10 min of hyperglycemic clamp test; ISI, insulin sensitivity index; clamp DI; clamp disposition index.

vary independently of mean and variance, given its definition formula. Indeed, AC_Var was not significantly correlated with DTW_Sev ($r = 0.06$; 95% CI: −0.20 to 0.32). Based on these rationales, we hypothesized that combining AC_Var with the DTW-based measures could improve the prediction of glucose handling capacity.

To test this hypothesis, we examined the relationship between DTW_Mod, DTW_Sev, AC_Var, DI, and diabetes diagnosis (Fig. 1c–h). Given the strong correlation between AC_Mean and AC_Var ($r = -0.74$; 95% CI: −0.85 to −0.60), we focused on AC_Var. As DTW_Low, DTW_Mod and DTW_Sev sum to 1, we excluded DTW_Low. We chose DI because it effectively reflects glucose disposal capacity and predicts the development of T2DM[47]. Clustering analysis based on DTW_Mod, DTW_Sev and AC_Var (Fig. 1c) revealed four distinct groups: cluster 1 (low DTW_Sev, low AC_Var), cluster 2 (low DTW_Sev, high AC_Var), cluster 3 (high DTW_Sev, low AC_Var) and cluster 4 (high DTW_Sev, high AC_Var). Participants in cluster 3 had significantly lower oral DI compared to cluster 1 (Fig. 1d), while those in cluster 2 had significantly reduced clamp DI compared to cluster 1, suggesting that elevated DTW_Sev (cluster 3) or AC_Var (cluster 2) is associated with reduced DI. As predicted, cluster 4 had significantly lower values for both oral DI and clamp DI compared to cluster 1.

We next investigated the association between cluster assignment and diabetes diagnosis (Fig. 1e). Cluster 4 was enriched for impaired glucose tolerance (IGT) and T2DM, whereas cluster 1 contained predominantly individuals with NGT. Among those diagnosed with NGT, some individuals with high AC_Var were assigned to clusters 2 or 4. Within this NGT subgroup (NGT_2), clamp DI values were significantly lower than NGT individuals in cluster 1 (NGT_1) and comparable to those with IGT (Fig. 1f), suggesting that AC_Var can identify individuals who are diagnosed as NGT based on FBG, HbA1c and OGTT, but whose disposition index is as low as that of IGT.

We then performed multiple regression analyses among oral DI, clamp DI, and the CGM-derived indices (Fig. 1g–i). R^2 of the models predicting oral DI and clamp DI from DTW_Mod, DTW_Sev, and AC_Var were 0.24

and 0.14, respectively. AC_Var showed significant independent negative correlations with both oral DI ($P = 0.029$) and clamp DI ($P = 0.010$), suggesting that AC_Var contributes to the prediction of DI independently of DTW-based measures. AC_Var also had a negative correlation with clamp DI that was statistically significant ($P = 0.048$) independent of CGM_Std (Fig. 1i), which was significantly correlated with clamp DI (Fig. 1b). R^2, the adjusted coefficient of determination (Adj R^2), and the Akaike Information Criterion (AIC) of the model predicting clamp DI from CGM_Std and AC_Var were 0.18, 0.15, and 583, respectively. Of note, R^2, AdjR^2, and AIC of the model predicting clamp DI from oral DI were only 0.15, 0.14, and 583, respectively. Moreover, R^2, AdjR^2, and AIC of the model predicting clamp DI from FBG, HbA1c and PG120 were only 0.09, 0.046, and 591, respectively. Collectively, we conclude that combining AC_Var with conventional CGM-derived indices can increase the accuracy of predicting DI.

Relationship among clinical parameters

To provide an overview of the relationship among indices derived from OGTT, clamp tests, CGM, and other clinical parameters, we constructed a correlation network (Fig. 2a). AC_Mean and AC_Var showed significant correlations with some insulin-related indices (blue nodes), but relatively weak associations with other parameters (red, magenta and green nodes). After Benjamini-Hochberg correction for multiple comparisons ($Q < 0.05$), AC_Var retained significant correlations with oral DI and clamp DI (Supplementary Data 1). In contrast, DTW_Sev and CGM_Mean correlated significantly with FBG, HbA1c, and PG120. The weak correlations between AC_Var and FBG, HbA1c, and PG120 suggest that it captures different aspects of glucose regulation that cannot be captured by conventional diabetes diagnosis methods.

To assess multicollinearity of the variables, we calculated variance inflation factors (VIF) for these variables. Since the purpose of this study was to estimate glucose handling capacities from relatively easy-to-measure indices, we included only CGM-derived indices, indices from a single blood test, and those from physical measurements as the input variables (CGM_Mean, CGM_Std, CONGA, LI, JINDEX, HBGI, GRADE, MODD, MAGE, ADRR, MVALUE, MAG, DTW_Mod, DTW_Sev, AC_Mean, AC_Var, TC, TGs, LDL-C, HDL-C, FBG, HbA1c, BMI, abdomen circumference (Acir), body fat percentage, SBP, and DBP) (Fig. 2b, Methods). We removed the variable with the highest VIF one by one until the VIF of all variables were less than 10, resulting in 18 variables (DBP, SBP, AC_Var, AC_Mean, HBGI, MAG, GRADE, DTW_Mod, ACir, BMI, MODD, FBG, HbA1c, TG, HDL-C, LDL-C, body fat percentage, MVALUE) (Fig. 2c). AC_Mean and AC_Var were included in these variables. Even when excluding measures until the VIF of all variables was less than 5, AC_Mean was included in the remaining variables (Supplementary Fig. 1b), suggesting that the autocorrelation function of glucose levels has relatively low multicollinearity with other measures.

Prediction of glucose control abilities

To identify key variables for DI prediction, we used PLS regression with VIP scores[37] (Fig. 2d, e) and Lasso regression[39] (Fig. 2f–k). PLS regression has been used for datasets with mutually correlated input variables and output variable, and important predictors can be estimated by VIP[37]. Lasso employs L1 regularization, which leads to models with fewer parameters, and has been used to select useful features to predict DM from numerous input variables[38]. Cross-validation identified two optimal PLS components, with AC_Var and several other CGM-derived measures having VIP scores >1 (Fig. 2d, e). The leave-one-out cross validation indicated that the optimal regularization coefficients of lasso (Lambda) were 0.061 for oral DI and 3.49 for clamp DI (Fig. 2f, g). At these Lambdas, the coefficients of AC_Var were estimated as non-zero coefficients for both oral DI and clamp DI (Fig. 2h–k). Collectively, these results indicate that CGM-derived indices, including AC_Var, contribute to the prediction of DI.

To further investigate the predictive potential of glucose dynamics for glucose regulatory capacity, we implemented random forest models using the indices shown in Fig. 2b. Decreases in insulin secretion and insulin sensitivity have been reported to start years before diabetes development and to be present in the pre-diabetes stage[3], and in this study, some individuals diagnosed with NGT had relatively low I.I. and composite index (Supplementary Fig. 2a). We defined decreased glucose control abilities based on established parameters: I.I. <0.4, composite index <3.0, FBG > 110 mg/dL or PG120 > 140 mg/dL (Supplementary Fig. 2a, b). These thresholds are consistent with previously reported abnormal ranges in the literature[48–50].

The maximum leaf nodes of the 6 and the features with AC_Mean and AC_Var provided the relatively better performance for predicting decreased glucose control abilities, with the accuracy and F1 scores of 0.73 and 0.51, respectively (Supplementary Fig. 2c). Feature importance analysis showed that AC_Mean was a stronger predictor than conventional markers including FBG (Supplementary Fig. 2d). The Boruta algorithm[43], which uses strict feature selection criteria by comparing shadow features, confirmed the statistical significance of AC_Mean in predictive accuracy ($Q < 0.05$) (Supplementary Fig. 2d).

To validate the importance of AC_Mean and AC_Var in predicting decreased glucose control abilities, we also performed a logistic regression analysis with L1 regularization (Supplementary Fig. 2e–g). The leave-one-out cross validation indicated that the optimal regularization coefficients, lambda, was 18.5 (Supplementary Fig. 2e, dashed line). At this lambda, the coefficient of AC_Var was estimated as non-zero coefficient (Supplementary Fig. 2f, g). Collectively, these results indicate that including AC_Mean or AC_Var alongside conventional indices improves the accuracy of identifying individuals with decreased glucose handling capacities.

Validation of the characteristics of AC_Mean and AC_Var using an independent dataset

To validate the utility of AC_Mean and AC_Var in predicting glucose control capacity, we analyzed an independent dataset[14] of 57 participants who were free from prior diabetes diagnosis, with 5 individuals meeting the criteria for T2DM, 14 having pre-DM, and the remaining participants having NGT (Methods). A correlation network showed that AC_Mean and AC_Var were significantly correlated with insulin sensitivity (SSPG, $r = -0.36$; 95% CI: −0.57 to −0.09 and $r = 0.48$; 95% CI: 0.23 to 0.68, respectively), while showing modest correlations with other CGM-derived indices (Fig. 3a). These associations remained significant ($Q < 0.05$) after Benjamini–Hochberg correction for multiple comparisons (Supplementary Data 2). These results were consistent with the result that AC_Var was significantly correlated with k_4, which corresponds to insulin sensitivity (Fig. 1b).

The assessment of multicollinearity using VIF revealed that AC_Mean exhibited relatively low multicollinearity with other indices (Fig. 3b, c), consistent with findings from previous analyses (Fig. 2b, c). The VIF of the indices calculated using the previously reported dataset (VIFp) and those calculated using the dataset obtained in this study (VIFt) were statistically significantly correlated (Fig. 3d), confirming the reproducibility of the relationships between the clinical parameters.

PLS regression identified AC_Var as an important predictor of SSPG (VIP > 1; Fig. 3e). Similarly, Lasso regression with leave-one-out cross-validation (Lambda = 0.69) confirmed AC_Mean and AC_Var as significant predictors of SSPG (Fig. 3f–h).

To investigate the effects of meal composition on AC_Mean and AC_Var, we calculated these indices using the previously reported CGM data that were collected after consuming standardized meals[14] (Supplementary Fig. 3). The standardized meals were about the same in calories, but differed in the amounts of proteins, fat, and fiber: cornflakes and milk (Cereal) were low in fiber and high in sugar, peanut butter sandwiches (Bread and PB) were high in fat and high in protein, and PROBAR protein bars (Bar) were moderate in fat and protein. The previous study indicated that DTW_Low, DTW_Mod, and DTW_Sev were able to capture the differences in glucose fluctuations due to different meals (Supplementary

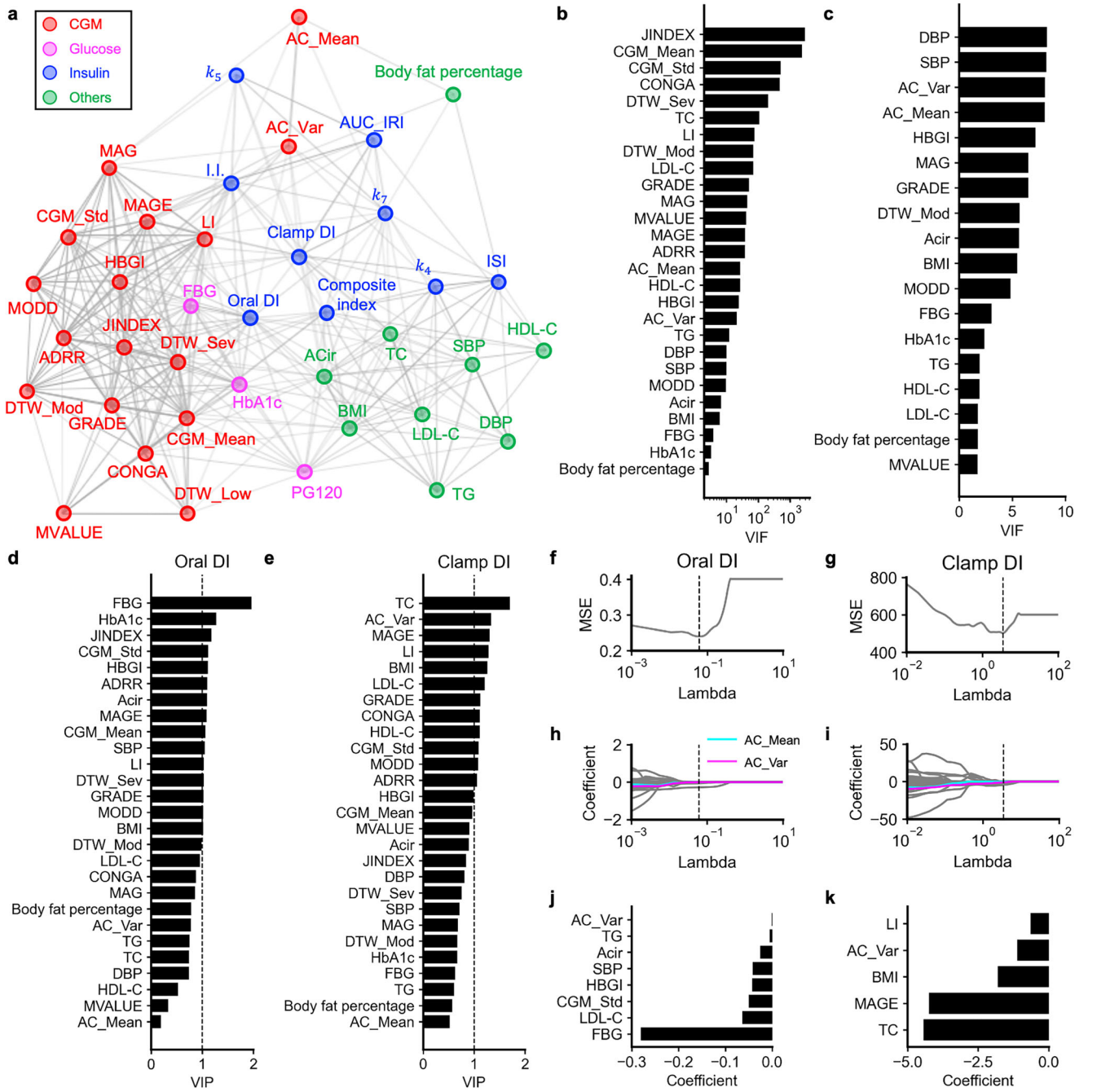

Fig. 2 | Relationship between clinical measures. a A spring layout of the correlation network of CGM-derived indices (red), blood glucose-related indices (magenta), insulin sensitivity, secretion, and clearance-related indices (blue); and other clinical measures (green). Relationships with absolute Spearman's correlation coefficients of 0.25 or higher are connected with edges. The width of the edges is proportional to the corresponding correlation coefficient. **b** VIF of all variables. **c** VIF of each variable remaining after removing the variable with the highest VIF one by one until the VIF of all variables are less than 10. **d**, **e** VIP scores from PLS regression for predicting (**d**) oral DI and (**e**) clamp DI. Dotted lines indicate significance threshold (VIP ≥ 1). **f**, **g** Relationship between regularization coefficients (Lambda) and the mean squared error (MSE) based on the leave-one-out cross-validation in predicting (**f**) oral DI and (**g**) clamp DI. Dotted vertical lines indicate optimal lambda values. **h**, **i** Lasso regularization paths along the Lambda in predicting (**h**) oral DI and (**i**) clamp DI. Cyan, magenta, and gray lines indicate the estimated coefficients of AC_Mean, AC_Var, and the other input variables, respectively. Dotted vertical lines indicate the optimal Lambda. **j**, **k** Estimated coefficients at the optimal Lambda in predicting (**j**) oral DI and (**k**) clamp DI. Only variables with non-zero coefficients are shown. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; PG120, plasma glucose concentration at 120 min during the oral glucose tolerance test; I.I., insulinogenic index; oral DI, oral disposition index; AUC_IRI, area under insulin curve during the first 10 min of hyperglycemic clamp test; ISI, insulin sensitivity index; clamp DI; clamp disposition index, VIF; variance inflation factor, VIP; variable importance in projection.

Fig. 3a)[14]. By contrast, one-way analysis of variance for testing the significance of differences in AC_Mean and AC_Var for each meal showed no significant difference (Supplementary Fig. 3b), suggesting that AC_Mean and AC_Var were more robust to meal types.

AC_Mean and AC_Var reflect changes in glucose dynamics when DI and insulin clearance change simultaneously

We next examined AC_Mean and AC_Var characteristics using simulated blood glucose data. We focused on DI and insulin clearance, as these

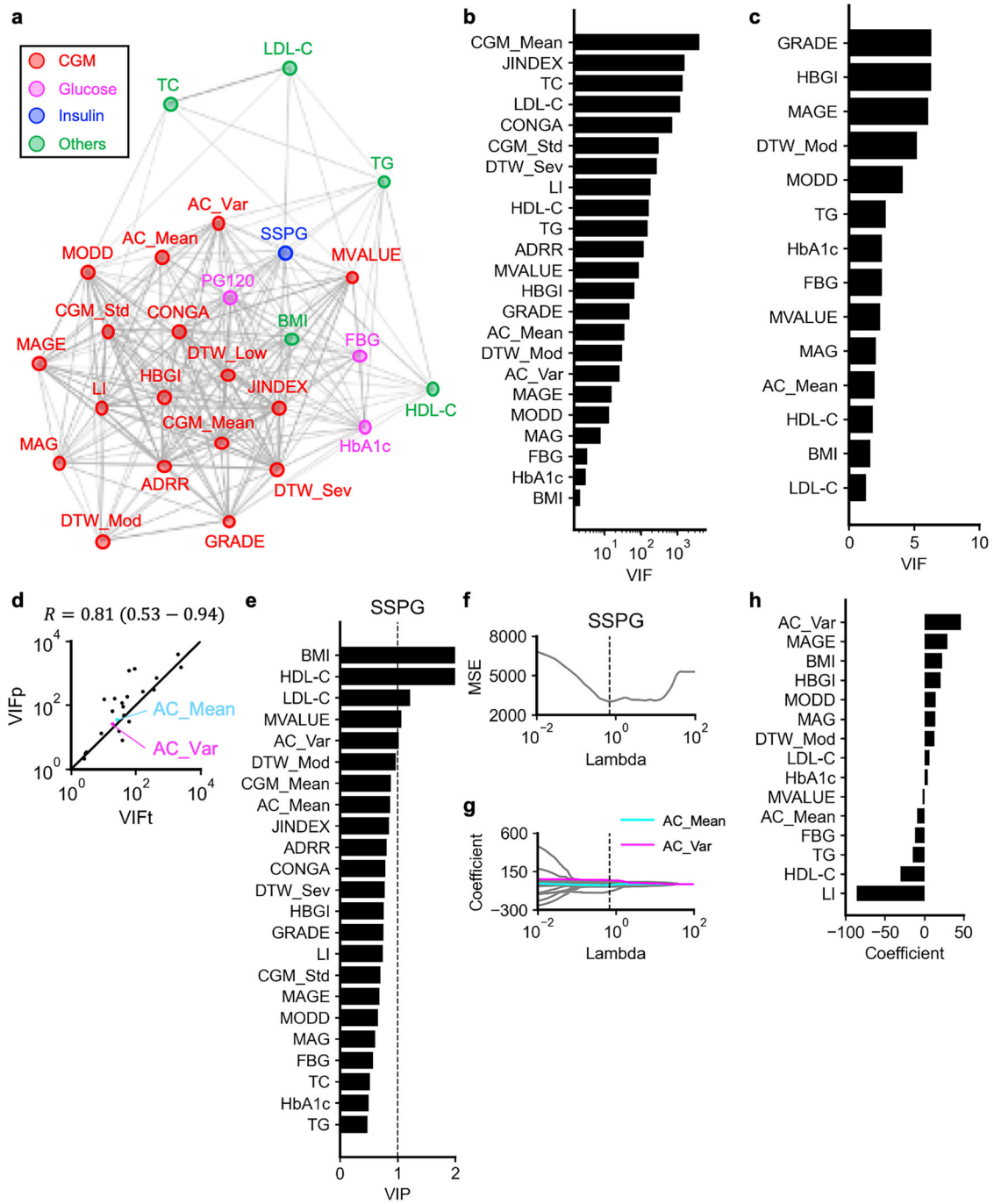

Fig. 3 | Validation of AC_Mean and AC_Var characteristics using an independent dataset[14]. **a** A spring layout of the correlation network of CGM-derived indices (red), blood glucose-related indices (magenta), insulin sensitivity-related index (blue); and other clinical measures (green). Relationships with absolute Spearman's correlation coefficients of 0.25 or higher are connected with edges. The width of the edges is proportional to the corresponding correlation coefficient. The analysis is based on data from 57 participants. **b** VIF of all variables. **c** VIF of each variable remaining after removing the variable with the highest VIF one by one until the VIF of all variables are less than 10. **d** Comparison of VIF values between the previously reported dataset (VIFp) and the current study dataset (VIFt). Points represent individual indices. Spearman correlation coefficient *R* is shown with 95% confidence intervals. **e** VIP scores from the PLS regression predicting SSPG. Dotted line indicates significance threshold (VIP ≥ 1). **f** Relationship between regularization coefficients (Lambda) and the mean squared error (MSE) based on the leave-one-out cross-validation in predicting SSPG. Dotted vertical lines indicate optimal lambda values. **g** Lasso regularization paths along the Lambda in predicting SSPG. Cyan, magenta, and gray lines indicate the estimated coefficients of AC_Mean, AC_Var, and the other input variables, respectively. Dotted vertical lines indicate the optimal Lambda. **h** Estimated coefficients with the optimal Lambda in predicting SSPG. Only variables with non-zero coefficients are shown. BMI, body mass index; TC, total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; FBG, fasting blood glucose; PG120, plasma glucose concentration at 120 min during the oral glucose tolerance test; SSPG, steady state plasma glucose; VIF, variance inflation factor; VIP, variable importance in projection.

parameters decrease simultaneously in the early stage of glucose intolerance[29]. For simulation of the mathematical model (see Methods), we used parameters reported as the mean values in NGT[31]. We changed DI ($k_{sec}k_{sen}$) and insulin clearance (k_{cle}) from one-half to twice the NGT's values (Fig. 4a). As $k_{sec}k_{sen}$ and k_{cle} increased, FBG levels remained unchanged (Fig. 4a, #α), but there was a point at which blood glucose levels decreased (Fig. 4a, #β) and a point at which blood glucose levels increased (Fig. 4a, #γ). As the pattern of blood glucose dynamics changed in this way,

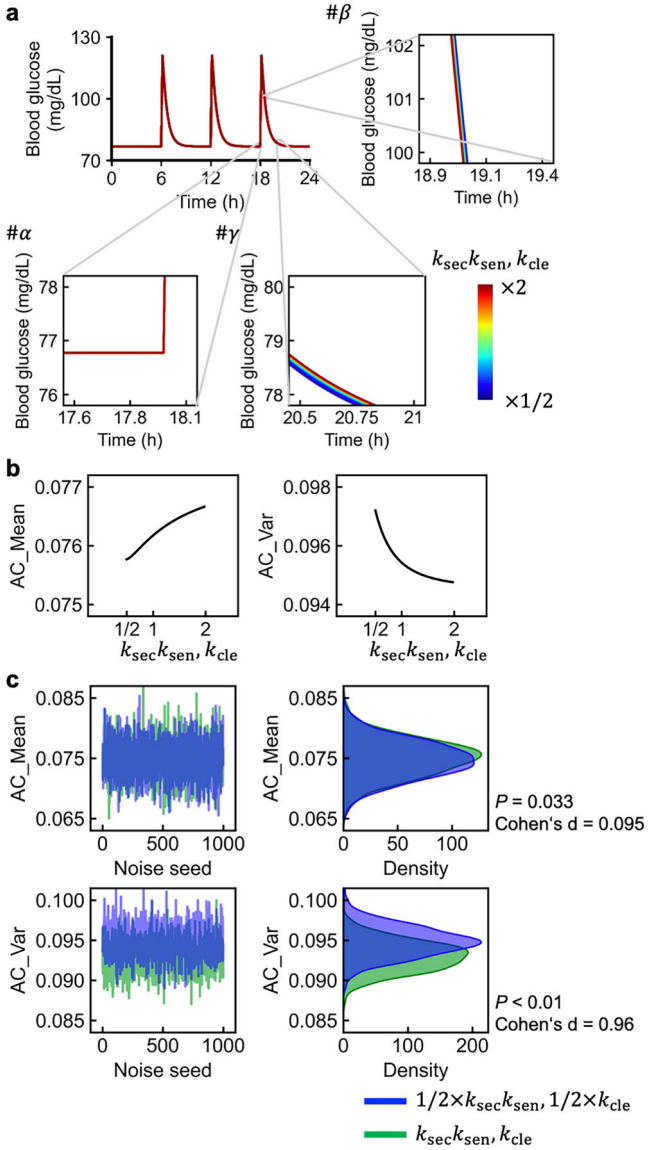

Fig. 4 | Characterization of AC_Mean and AC_Var using simulated glucose dynamics. a Twenty-four-hour simulated glucose concentration profiles colored according to parameter values of $k_{sec}k_{sen}$ and k_{cle}. #α-#γ show magnified views of highlighted regions. **b** The relationship between $k_{sec}k_{sen}$, k_{cle}, AC_Mean, and AC_Var. The horizontal axis represents the ratio of $k_{sec}k_{sen}$ and k_{cle} to the reported average values for healthy individuals. **c** AC_Mean and AC_Var simulated from the glucose concentration with Gaussian white noise. AC_Mean and AC_Var calculated in each trial and the distributions of AC_Mean and AC_Var are shown. The green is simulated using the parameters reported as the mean values for healthy individuals. The blue is simulated using half the mean values. The *P* values are for testing the hypothesis of no difference between the two groups.

AC_Mean increased and AC_Var decreased (Fig. 4b), consistent with the results that AC_Mean was positively correlated with insulin clearance, and AC_Var was negatively correlated with DI and insulin clearance (Fig. 1b). We validated these relationships using an independent mathematical model describing both single meal and daily glucose dynamics[32], which showed similar directional changes in AC_Mean and AC_Var with increasing insulin secretion, sensitivity, and clearance (Supplementary Fig. 4a, b).

To assess the noise sensitivity of AC_Mean and AC_Var, we compared blood glucose dynamics simulated using the mean values of $k_{sec}k_{sen}$ and k_{cle} at NGT with those simulated using the half values of the parameters. Applying Gaussian white noise with the mean of 0 and the variance of 1 (Supplementary Fig. 4c, d), followed by the calculation of AC_Mean and AC_Var was repeated 1000 times. Since the exact modeling of the statistical properties of CGM sensor errors is difficult[51], we only investigated the properties of AC_Mean and AC_Var at different white noise magnitudes. Of note, the blood glucose difference due to the differences in $k_{sec}k_{sen}$ and k_{cle} was at most 0.45 mg/dL, and the variance of the difference was only 0.014. Even if the applied noise is greater than the difference between the two groups, AC_Var in particular could distinguish the two groups (Fig. 4c, $P<0.01$). Cohen's d of AC_Var was larger than that of AC_Mean (Fig. 4c), suggesting that AC_Var was more robust to noise than AC_Mean. Of note, Cohen's d quantifies the size of the difference between two groups, and conventionally Cohen's d values of 0.2, 0.5 and 0.8 represent small, medium and large effect sizes, respectively. A larger value of Cohen's d indicates a larger difference between groups. The observed correlation between AC_Var and DI, which is stronger than the correlation between AC_Mean and DI shown in Fig. 1b, is consistent with the ability of AC_Var to discriminate between differences in DI despite added noise, as shown in Fig. 4c.

As the variance of the noise was reduced, the Cohen's d of both AC_Mean and AC_Var became larger (Supplementary Fig. 4e–h). As the measurement period was increased from 24- to 72-h (Supplementary Fig. 4i), or as the measurement interval was reduced from every 5 min to every 1 min (Supplementary Fig. 4j), the Cohen's d of both AC_Mean and AC_Var became larger. Under all conditions (Fig. 4c, Supplementary Fig. 4d–j), Cohen's d of AC_Var was larger than that of AC_Mean.

Web application for calculating CGM-derived indices

To easily calculate CGM-derived indices, we developed a web application (https://cgm-ac-mean-std.streamlit.app/)[26] that calculates CGM_Mean, CGM_Std, AC_Mean, and AC_Var (Supplementary Fig. 5). This application was implemented in Streamlit. In using this application, glucose should be measured every 5 min. The application can also run on a local machine using the code in GitHub repository (https://github.com/HikaruSugimoto/CGM_AC)[26].

Discussion

Here we found that AC_Var was significantly correlated with both insulin clearance and DI and that AC_Var could identify a subgroup of NGT individuals with reduced DI comparable to those with IGT. Previous studies have shown that insulin clearance is a predictor of the development of T2DM in non-DM individuals[1], while DI has been shown to predict T2DM progression independently of FBG and PG120[2]. Collectively, these findings suggest that AC_Var can identify abnormalities in glucose dynamics even in a predominantly NGT population, potentially serving as an alternative to single-point blood tests or OGTT, which have been shown to be inadequate[6–8] or inconvenient[9] for pre-diabetes screening.

We used several analytical approaches including multiple linear regression, PLS regression, Lasso regression, random forests, and logistic regression with L1 regularization to predict DI, insulin secretion and insulin sensitivity. Although DI has been identified as a predictor of the development of T2DM[2] and reduced insulin sensitivity and secretion are known to precede the onset of T2DM[3], accurate quantification of these parameters requires extensive testing. Our predictive models only included indices derived from a single-point blood test, physical examinations, and CGM, which are relatively easy to measure. Given that the predictive performance of the linear regression model with CGM_Std and AC_Var as input variables and clamp DI as the objective variable was about the same as that of predicting clamp DI from oral DI, these relatively easy-to-measure indices can be alternatives to OGTT and clamp tests in screening protocols.

Our analysis focused primarily on the mean and variance of the autocorrelation function at lags 1-30, established CGM-derived indices[21], and three previously reported indices for identifying abnormal glucose regulation[14]. However, the mean and the variance of the autocorrelation function at lags 1–2 (Supplementary Fig. 1a) were also significantly correlated with oral DI ($r = -0.29$; $P = 0.02$ and $r = 0.29$, $P = 0.02$, respectively), and other CGM-derived indices of glycemic variability have been reported[27,52–63]. It is necessary to investigate these other CGM-derived

indices comprehensively to determine the extent to which glucose handling capacities can be characterized from CGM in the future.

The current study has several limitations. This study focused on predicting current glucose tolerance from CGM data. Future work using longitudinal CGM data to predict long-term glycaemic outcomes may broaden the scope and applicability of our methods. Although we analyzed both Japanese and American datasets, CGM data may be influenced by ethnicity and lifestyle; therefore, larger and more diverse populations are needed. While AC_Var showed significant correlations with insulin sensitivity indices in both Japanese (k_4) and American (SSPG) datasets, the American dataset did not include the DI, which is the product of insulin sensitivity and insulin secretion. The relationship between AC_Var and DI in the American population requires further validation.

The physiological meaning of AC_Mean and AC_Var may appear ambiguous at first glance. However, as shown in Fig. 1a, it is hypothesized that the autocorrelation of glucose levels decreases significantly with increasing lag in individuals with rapid glucose fluctuations, resulting in smaller AC_Mean and larger AC_Var values. Furthermore, as shown in Figs. 2–4, lower AC_Mean and higher AC_Var values correspond to decreased disposition index or insulin clearance, suggesting that the autocorrelation function of glucose levels serves as a physiological indicator of glucose regulatory capacity.

The robustness of AC_Mean and AC_Var to meal types could be perceived as a positive attribute in certain scenarios. For example, in the context of estimating glucose handling capacities, the greater robustness means that these indices are less sensitive to variations induced by different meal types, potentially providing more consistent or reliable insights into estimating glucose handling capacities under different dietary conditions. However, the assessment of whether this robustness is advantageous or disadvantageous depends on the study objectives and practical applications, and the effect of meal type on glucose dynamics requires further investigation.

Although the correlation coefficients in this study may appear modest, it is important to note that this dataset mainly includes individuals with NGT, which limits variability in glucose handling capacities. Despite these constraints, this study revealed meaningful statistically significant relationships. While the relationship between clamp DI and oral DI, an index designed to estimate clamp DI from OGTT and reported to correlate well with clamp DI, had an R^2 of only 0.15, the relationship between clamp DI and CGM-derived indices had an R^2 of 0.18, indicating the potential of CGM-derived indices in estimating glucose handling capacities. Furthermore, as shown in our mathematical simulations (Fig. 4), AC_Mean decreases monotonically and AC_Var increases monotonically as the disposition index decreases. These relationships suggest that our findings should be reproducible and potentially more significant in populations with a wider range of glucose-handling capacities. In addition, our analysis was limited by the use of only three days of CGM data, and the CGM device used in the validation set had a higher mean absolute relative difference (MARD) compared with contemporary models. Nevertheless, CGM-derived indices showed significant correlations with insulin sensitivity or DI. Given that mathematical modeling simulations suggest that longer monitoring periods may improve the accuracy of AC_Var measurements, future studies using longer monitoring periods and more advanced CGM devices with improved accuracy may provide a more accurate estimate of glucose regulatory capacity.

Regarding test-retest reproducibility (i.e., repeated measurements in the same individuals), we acknowledge that we do not have data from multiple CGM measurement periods in the same individuals, which would be required to directly assess this type of reproducibility. However, we can speculate the robustness of these indices in several ways. First, our results show that CGM-derived indices can predict clamp DI with comparable or better accuracy than predictions using oral DI or conventional clinical markers (FBG, HbA1c, and 2-h OGTT glucose). If the CGM-derived indices had poor reproducibility, we would expect their predictive performance to be inferior to these OGTT-derived measures. Furthermore, the reproducibility of our findings across different populations provides additional confidence in these indices. The relationships we observed between CGM-derived indices and measures of glucose handling capacity were consistently reproduced in both Japanese and American datasets, despite differences in ethnicity, lifestyle, and CGM devices used.

In conclusion, the current study demonstrated that CGM-derived indices can predict DI beyond conventional markers such as FBG and HbA1c. CGM-derived DI (CGM DI) may serve as an alternative to the labor-intensive measurements involved in conventional DI assessment using OGTT (oral DI) or clamp tests (clamp DI) in screening protocols.

Data availability

The CGM data that support the findings of this study are freely available from the GitHub repository (https://github.com/HikaruSugimoto/CGM_AC). Previously reported CGM data[14] is publicly available and can be downloaded from https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.2005143. The source data for Figs. 1–4 is in https://zenodo.org/records/15067145[26]. All other data are available from the corresponding author on reasonable request.

Code availability

The code that calculates AC_Mean and AC_Var is also available from the repository (https://github.com/HikaruSugimoto/CGM_AC)[26] and the web application (https://cgm-ac-mean-std.streamlit.app/)[26].

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Author contributions

H.S. analyzed the data. H.S., K.H., T.N., T.Y., H.M., N.O-S., M.F., Y.H., K.S., W.O., and S.K. wrote the manuscript. T.N., T.Y., H.M., and N.O-S. conducted clinical examinations. W.O. and S.K. supervised the study.

Competing interests

The authors declare no competing interests.

Additional information

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