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Postprandial glucose profiles may reflect heterogeneity in insulin secretion and sensitivity in type 2 diabetes

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ABSTRACT

Background: Continuous glucose monitoring (CGM) reveals heterogeneity of postprandial glucose responses (PPGR), a key target for optimizing glycemic control in type 2 diabetes (T2D). We analyzed PPGR patterns to identify subtypes reflecting pathophysiological differences.

Methods: Cross-sectional CGM data from 100 individuals with T2D were collected over 4 h following a standardized meal consumed twice. Dynamic PPGR features—glucose peak, incremental area under the curve (iAUC), rise and fall rates, final vs. fasting glucose—were used for K-Means clustering, with stability assessed using a Random Forest classifier trained on the first meal. In 50 participants, postprandial plasma glucose and insulin were measured, and clinical/metabolic parameters compared across clusters using one-way ANOVA.

Results: Three CGM-defined PPGR clusters were identified. Cluster 1 ($n = 19$) showed the highest peak and iAUC, with post-meal glucose remaining persistently above baseline. Cluster 2 ($n = 56$) and 3 ($n = 25$) had lower peaks and iAUCs, but Cluster 3 exhibited higher rise and fall rates than Cluster 2. Clusters did not differ in age, sex, BMI, or diabetes duration, but metformin use was lower in Cluster 3. Cluster 1 showed significantly lower insulin secretion (HOMA2-B%: 77.42 ± 25.64 vs. 104.96 ± 43.94) and higher insulin resistance (HOMA-IR: 7.94 ± 3.27 vs. 4.84 ± 2.78) than Cluster 3, with intermediate values for Cluster 2, confirmed by postprandial indices. Cluster 3 had a higher early insulin response than Cluster 1 and 2 (60-min insulinogenic index: 1.67 ± 1.07, 0.84 ± 0.31, 0.84 ± 0.58, respectively; $p < 0.05$).

Conclusions: CGM-derived PPGR features could identify T2D subtypes with similar clinical profiles but distinct insulin secretion and sensitivity impairments, supporting targeted interventions.

1. Introduction

Type 2 diabetes (T2D) is a heterogeneous disease characterized by differences in pathophysiology as well as clinical and prognostic implications. Ahlqvist and colleagues [1] proposed a stratification of T2D into subgroups that capture this heterogeneity based on patient characteristics. This sub-grouping is driven by varying degrees of derangement in insulin secretion and insulin sensitivity, which progressively impair the regulation of plasma glucose concentrations and contribute to the wide variability in clinical presentation and long-term risk of complications [2,3].

Within this framework, growing efforts have been dedicated on identifying scalable and cost-effective tests capable of capturing the complexity of T2D subtypes. The postprandial glucose response (PPGR) is a well-established risk factor for the development of cardiovascular diseases and other diabetic complications, thus representing a relevant

Abbreviations: T2D, Type 2 Diabetes; CGM, Continuous Glucose Monitoring; PPGR, Postprandial Glucose Response; BMI, Body Mass Index; HOMA, Homoeostasis Model Assessment; iAUC, incremental Area Under the Curve; ISSI-2, Insulin Secretion-Sensitivity Index.

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therapeutic target [4,5]. Moreover, PPGR is emerging as a promising marker of phenotypic heterogeneity, having shown high variability in response to identical meals due to the influence of multiple factors such as genotype, insulin secretion and action, gut microbiota, hormones, diet and lifestyle habits [6,7].

So far, the PPGR has often been evaluated by measuring plasma glucose concentrations only at a few time points. An important limitation of this approach is that it does not allow disentangling of specific impairments in postprandial glucose dynamics that could capture differential plasma glucose profiles within and between individuals. Such variation in postprandial glucose profile can be modulated by meal composition and by underlying pathophysiological mechanisms and may have a significant impact on the daily glucose profile.

The use of continuous glucose monitoring (CGM) allows the assessment of the dynamic aspects of the PPGR, by enabling accessible time-series measurements of the interstitial glucose concentrations. A recent study used CGM-generated glucose curves obtained during a 16-point glucose tolerance test to identify metabolic subphenotypes of prediabetes characterized by specific impairments either of insulin sensitivity predominantly at the liver or at the muscle level or by a dysfunctional beta-cell or by an impaired-incretin action [8].

In a previous study from our group, CGM data from individuals at increased risk of T2D were fitted into a mechanistic model of postprandial glucose regulation in response to non-standardized breakfast meals, to define relevant parameters of postprandial glucose dynamics. By this approach, we found two distinct clusters of PPGR: one characterized by an early rise in glucose concentrations, and the other one by a flat and prolonged elevation of postprandial glucose concentrations, associated with increased insulin resistance [9]. These data support that PPGR dynamic profiles reflect key pathophysiological impairments underlying the clinical and prognostic characteristics of individuals with T2D. Identifying specific impairments using CGM may provide a simple approach to enable personalized therapeutic interventions for PPGR by targeting the specific underlying metabolic derangements and delaying disease progression. This may be particularly relevant for individuals with well-controlled T2D, as defined by HbA1c levels, in whom PPGR plays a major role than fasting hyperglycemia in the overall metabolic dysregulation [10,11]. Therefore, the primary aim of this study was to investigate glucose response patterns in individuals with well-controlled T2D based on HbA1c using CGM-derived dynamic parameters following a standardized meal. A further aim was to evaluate the association between distinct PPGR patterns and specific pathophysiological impairments relevant to diabetes progression and its long-term complications.

2. Research design and methods

2.1. Study participants

Patients with T2D attending the Diabetes Unit at the Federico II University Hospital were recruited in the DIABEST study. This cross-sectional study – registered in ClinicalTrials.gov (registration number: NCT06057246) – evaluates clinical and metabolic features of individuals with T2D associated with specific PPGR patterns. The diagnosis of T2D was based on HbA1c or plasma glucose, according to the American Diabetes Association [12]. Eligibility criteria included: age 30–70 years; HbA1c values $\leq$7.5% (58 mmol/mol); T2D on treatment with diet or diet plus metformin; Body Mass Index (BMI) between 20 and 39.9 kg/m^2. Exclusion criteria consisted of any acute or chronic condition that could interfere with the interpretation of the results (e.g., Celiac disease, liver failure, etc.), unwillingness to consume study-related foods or inability to complete the study protocol activities. All potential participants were screened for eligibility and given the opportunity to ask questions or express any concerns. The study protocol and the procedures for obtaining informed consent were reviewed and approved by the Federico II University Institutional Review Board (IRB Protocol #31/2023).

2.2. Study design

Each enrolled participant attended a first visit at the diabetes outpatient clinic where a fasting blood sample was withdrawn to measure glucose and insulin concentrations; thereafter, anthropometric measurements (i.e., weight, height and waist circumference) were performed and a CGM sensor (Dexcom One/Dexcom One$^+$) was placed. Each study participant received foods for the standardized meal, along with relevant instructions for at-home consumption. Each participant consumed the meal at home for the first time and was asked to repeat it with identical foods either at the clinic or once again at home after the 7-day observation period. For those repeating the meal at the clinic, venous blood samples were drawn before and up to 4 h after the meal, to evaluate postprandial plasma glucose and insulin concentrations while still wearing the CGM.

2.3. Standardized meal composition and instructions

The standardized meal, consumed either twice at home or once at home and once in the clinic, consisted of white bread (80 g), spreadable cheese (50 g), air-cured beef (50 g) and one apple (150 g). Its nutrient composition was as follows: total energy (TE) content 532 kcal, carbohydrates 68,4 g (49.3% TE), total fat 17.1 g (29.5% TE), protein 27.8 g (21.4% TE), and fiber 6 g. Participants were instructed to fast for a minimum of 8 h before consuming the standardized meal (during 15 to 20 min) and to avoid any food as well as any moderate to intense physical activity for 4 h afterward.

2.4. Fasting and postprandial biomarkers and indices of insulin secretion and sensitivity

Among the study participants repeating the standardized meal at the clinic, fasting blood samples were drawn from an antecubital vein 15 min and immediately before eating the standardized meal, via a catheter inserted into an antecubital vein, which remained in place throughout the 4-h testing period. Thereafter blood samples were collected 15 min after they started the consumption of the standard meal and then at 30, 60, 120, 180 and 240 min. EDTA-plasma samples were immediately placed on ice or refrigerated, then processed and aliquoted into microtubes.

Glucose was measured by enzymatic colorimetry using an oxidase method on an ABX Pentra 400 Autoanalyzer (ABX Diagnostics, Montpellier, France). Insulin was measured by electrochemiluminescence immunoassay method by ELISA (DIAsource ImmunoAssays, Nivelles, Belgium) on a Triturus Analyzer (Diagnostics Grifols, Barcelona, Spain). HbA1c was determined by High Performance Liquid Chromatography (HPLC). Fasting C-peptide was quantified using a chemiluminescent immunoassay (CLIA).

Homoeostasis model assessment (HOMA) 2, which estimates beta-cell function (HOMA2-B), was calculated utilizing plasma C-peptide concentrations by the HOMA calculator (University of Oxford, Oxford, UK) [13]. C-peptide measurements were used instead of insulin concentrations for better performance among individual with T2D. Insulin resistance (IR) was estimated using the HOMA-IR based on fasting insulin and glucose concentrations [14]. To estimate insulin secretion and sensitivity in the postprandial state (i.e., after the standardized meal) the insulin secretion-sensitivity index (ISSI-2) [15], the Matsuda index [16], and the Disposition Index [17] were calculated. Early postprandial insulin secretion was assessed by calculating the 60-min Insulinogenic Index [18], and the ratio of the 0–120 min incremental area under the curve (iAUC) of insulin to glucose after the standardized meal, calculated using the trapezoidal rule [19].

2.5. CGM-derived postprandial glucose response parameters

Raw CGM glucose data collected every 5 min were utilized for the

evaluation of the 4-h glucose response to the standardized meal. Glucose values from time 0 (i.e., the reported start time of the meal in the food record) to 240 min post-meal, represented the 4-h PPGR.

The PPGR feature set comprised key descriptive metrics capturing glucose dynamics, including the peak concentration, $iAUC_{0\text{-}4h}$, the average rates of glucose rise and fall, and the capacity of glucose concentrations to return to baseline values within the monitored postprandial window.

The glucose peak (Eq. 1) is defined as the maximum value of the smoothed glucose concentration over the time series, capturing the highest postprandial glucose excursion. The glucose time series data were smoothed using a Savitzky-Golay filter ($G(t)_{smooth}$), which performs local polynomial regression to preserve important features such as peaks while reducing noise. Specifically, a third-order polynomial was fitted within a moving window of length 12 data points.

$$G_{peak} = \max\left(G(t)_{smooth} \right) \tag{1}$$

The iAUC, described in Eq. 2, quantifies the total positive glucose response above baseline by summing trapezoidal areas between consecutive time points, with negative increments truncated to zero to exclude excursions below baseline.

$$iAUC = \sum_{k=1}^{N-1} \frac{max(G_k - G_{baseline}, 0) + \max(G_{k+1} - G_{baseline}, 0)}{2} (t_{k+1} - t_k) \tag{2}$$

The mean rates of glucose rise (Eq. 3) and fall (Eq. 4) were calculated as the average discrete derivatives before and after the peak, respectively.

$$R_{rise} = \frac{1}{N_{rise}} \sum_{i=1}^{N_{rise}} G_{smooth}(t_{i+1}) - G_{smooth}(t_i), \quad t_i < t_{peak} \tag{3}$$

$$R_{fall} = \frac{1}{N_{fall}} \sum_{j=1}^{N_{fall}} G_{smooth}(t_{j+1}) - G_{smooth}(t_j), \quad t_j > t_{peak} \tag{4}$$

Finally, the return to baseline capability (G_δ, Eq.5) measures the mean difference between the last five glucose points and the baseline value, providing an indication of how fully glucose concentrations normalize by the end of the monitoring period.

$$G_\delta = \frac{1}{5} \sum_{k=N-4}^{N} G(t_k) - G_{baseline} \tag{5}$$

2.6. Data processing and statistical analysis

Participants were clustered based on the dynamic parameters derived from 4-h glucose response time-series following the standardized meal consumed at home. A K-Means clustering algorithm was applied to the scaled feature data to partition the participants into distinct groups. The optimal number of clusters was determined by evaluating standard validation metrics, such as the Silhouette index and the Davies-Bouldin index. Data processing was performed using Python (version 3.10). Associations between identified clusters of PPGR and clinical or metabolic features were evaluated using one-way analysis of variance (ANOVA). When appropriate, analyses were repeated using analysis of covariance (ANCOVA) to adjust for metformin use and HbA1c. Post hoc pairwise comparisons between clusters were performed using linear models, with adjustment for multiple testing using the Holm method. A two-sided P value <0.05 was considered statistically significant.

To assess the day-to-day reproducibility of glucose response dynamics, parameters were extracted from the standardized meals consumed in two separate occasions from each participant. Participants exhibiting highly variable responses between the two standardized meals were identified as outliers and excluded from further analyses, as this variability was visually determined to come from measurement noise. This was done by calculating the difference in feature values between the two meals for each participant, standardizing these differences using Z-scores. Participants with a scaled difference exceeding a threshold of $|Z| > 2$ (two standard deviations) for any of the selected features were removed.

To investigate the reproducibility of postprandial glucose responses and extracted features, we assessed intraclass correlation coefficients (ICC) across the repeated standardized meals.

2.7. Reproducibility of postprandial glucose response clusters

To assess how consistently participants were classified into the same cluster on different occasions, a Random Forest classifier was trained to predict cluster membership. The model was trained using the feature data from the first at-home consumption of the standardized meal as predictors, and the derived cluster labels as the outcome. The trained model was then applied to predict cluster membership for each participant based on the second consumption of the standardized meal. Reproducibility was assessed using classification quality metrics including accuracy, precision, recall, and F1-score. Accuracy reflects the ratio of correct classifications, precision represents the proportion of correctly classified positive cases among all positive predictions, recall (sensitivity) indicates the proportion of correctly classified positive cases among all true positive cases and F1-score is the harmonic mean of precision and recall [20].

3. Results

A total of 100 participants (69 men and 32 postmenopausal women) consumed the standardized meal for the first time at home under standardized conditions, and repeated the meal on a second occasion either at home (n = 50) or at the clinic (n = 50). Participant flow chart is available in Supplemental Fig. S1.

There were no differences in the characteristics between the overall study population and the subgroup of participants who repeated the standardized meal in the clinic while undergoing postprandial blood sampling (Supplemental Table S1).

3.1. Clusters of CGM-defined postprandial glucose response

Three distinct clusters of the 4-h glucose response were identified in

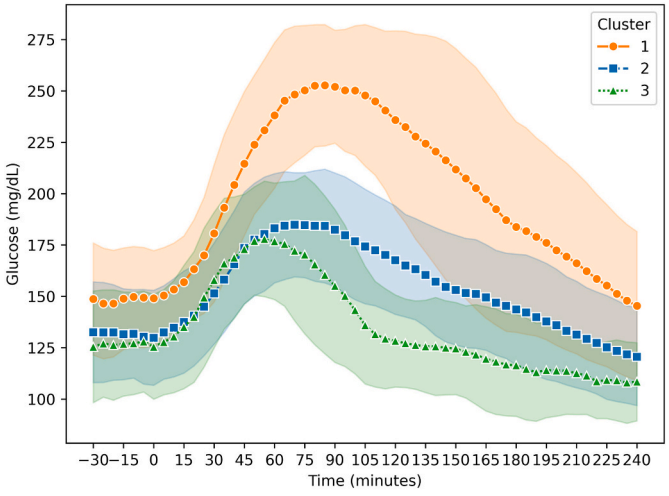

Fig. 1. Clusters of CGM-defined glucose response following the standardized meal consumed at home (n = 100). Dotted lines represent the mean glucose response and shaded areas indicate one standard deviation of the population mean at each time point.

the overall study population ($n = 100$) based on CGM-derived parameters following at-home consumption of the standardized mixed meal (Fig. 1).

Cluster 1 ($n = 19$) was characterized by the highest peak and $iAUC_{0\text{-}4h}$ values, with glucose concentrations remaining above baseline for the entire postprandial period. The other two Clusters (2 and 3) exhibited similar lower peak and $iAUC_{0\text{-}4h}$ values, but differed in their dynamic parameters, with Cluster 3 ($n = 56$) showing higher rates of glucose rise and fall compared to Cluster 2 ($n = 25$), and a lower difference between average glucose concentrations measured at 4 h after the meal and at fasting (glucose delta) (Table 1).

The overall glucose curve demonstrated moderate-to-good reproducibility throughout the 4-h period (ICC range: 0.65–0.78). In addition, agreement between postprandial glucose levels measured by CGM and those obtained by plasma samples at selected time points was consistently moderate to good (data not shown). Among the derived PPGR features used for clustering, glucose peak exhibited good reproducibility (ICC ≈ 0.69), while the rates of rise and fall were relatively more stable than summary measures such as iAUC (ICC ≈ 0.35–0.45). However, averaging repeated measurements improved the reproducibility of all CGM-derived postprandial glucose parameters (ICC up to ~0.8). Cluster stability analysis revealed moderate reproducibility of cluster assignments across repeated meals. The overall weighted F1-score (0.65) and accuracy (0.66) reflected moderate consistency in cluster structure when predicting membership on repeated meal responses.

3.2. Associations between clusters of CGM-defined postprandial glucose response and clinical and metabolic features

As reported in Table 2, there were no statistically significant differences among the three clusters of CGM-defined PPGR in terms of mean age, diabetes duration, and anthropometric measures (BMI and waist circumference) but metformin use was the lowest in Cluster 3.

Individuals in Cluster 1 exhibited the highest HbA1c concentrations, but the difference with the other two clusters, although statistically significant, was small. Indeed, HbA1c concentrations across all clusters remained within a narrow range consistent with good to optimal glycemic control – from 6.7 ± 0.6% (50 ± 6 mmol/mol) in Cluster 1 to 6.3 ± 0.4% (45 ± 4 mmol/mol) in Cluster 3. The proportion of individuals taking metformin was similar in Cluster 1 (68%) and 2 (75%), but significantly lower in Cluster 3 (20%). The HOMA indices of insulin secretion (HOMA2-B) and resistance (HOMA-IR) showed a progression across clusters. Indeed, Cluster 1 exhibited the lowest beta-cell function and highest insulin resistance, Cluster 3 had the highest beta-cell function and lowest insulin resistance, whereas Cluster 2 displayed intermediate profiles of both features. However, only comparisons between the extremes (i.e., Cluster 1 vs. Cluster 3) reached statistical significance, persisting after adjustment for metformin use by covariance analysis ($p = 0.018$ and $p = 0.021$ for HOMA2-B and HOMA-IR, respectively), but attenuated after adjustment for HbA1c ($p = 0.278$ and $p = 0.062$, respectively).

Table 1
Parameters of the CGM-defined postprandial glucose response by Cluster ($n = 100$).

	Cluster 1 N = 19	Cluster 2 $N = 56$	Cluster 3 $N = 25$
Peak smooth (mg/dL)	265.8 ± 28.6	193.1 ± 25.8*	186.5 ± 30.3*
$iAUC_{0\text{–}4h}$ (mg/dL·min)	8256 ± 1254	4261 ± 1463*	3481 ± 1415*
Mean rate of rise (mg/dL/min)	0.006 ± 0.002	0.005 ± 0.002	0.008 ± 0.002*†
Mean rate of fall (mg/dL/min)	−0.003 ± 0.002	−0.003 ± 0.001	−0.007 ± 0.002*†
Glucose delta (mg/dL)	95.4 ± 27.6	40.1 ± 21.3*	7.1 ± 15.3*†

Legend: *$p < 0.05$ vs. Cluster 1; † $p < 0.05$ vs. Cluster 2 according to Bonferroni post-hoc test.

Table 2
Metabolic and clinical features of the participants (n = 100) across clusters of CGM-defined postprandial glucose response.

	Cluster 1 $N = 19$	Cluster 2 $N = 56$	Cluster 3 $N = 25$
Female *n* (%)	5 (26)	17 (30)	10 (40)
Age (years)	59.2 ± 7.2	60.9 ± 6.7	62.8 ± 3.9
BMI (Kg/m^2)	30.0 ± 3.2	30.2 ± 5.4	30.3 ± 4.3
Waist Circumference (cm)	103.0 ± 8.6	101.9 ± 13.1	101.6 ± 11.5
Diabetes duration (years)	7.1 ± 5.8	4.5 ± 4.3	4.2 ± 4.8
HbA1c % (mmol/mol)	6.7 ± 0.6 (50 ± 6)	6.4 ± 0.5 (46 ± 5)*	6.3 ± 0.4 (45 ± 4)*
Metformin use *n* (%)	13 (68)	42 (75)	5 (20)*†
HOMA2-B%	77.42 ± 25.64	94.46 ± 31.59	104.96 ± 43.94*
HOMA-IR	7.94 ± 3.27	5.99 ± 3.36	4.84 ± 2.78*

Legend: *p < 0.05 vs. Cluster 1; † p < 0.05 vs. Cluster 2 according to Bonferroni post-hoc test. The Homeostasis Model Assessment (HOMA) indices are dimensionless.

3.3. Postprandial plasma glucose and insulin responses across clusters of CGM-defined postprandial glucose response

The subgroup of 50 participants who repeated the standardized meal at the clinic and underwent postprandial blood sampling exhibited a CGM-defined cluster distribution ($n = 13$ in Cluster 1; $n = 27$ in Cluster 2; $n = 10$ in Cluster 3) consistent with that of the overall study population. Furthermore, in this subgroup, the CGM-defined glucose responses following the first at-home consumption of the standardized meal showed consistent PPGR shapes (Supplemental Fig. S2) and relative CGM-derived dynamic parameters (Supplemental Table S2) compared with those assessed in the overall study population.

Plasma glucose and insulin responses for participants grouped into the three clusters are shown in Supplemental Fig. S3 and Supplemental Fig. S4. Glucose concentrations in Cluster 1 were significantly higher than in Cluster 3 at all time points and higher than in Cluster 2 at 30 and 120 min (Supplemental Fig. S3). Plasma glucose response also differed between Clusters 2 and 3 at later time points, with glucose concentrations in Cluster 3 being significantly lower than in Cluster 2 from 120 to 240 min.

Regarding postprandial plasma insulin concentrations, they were significantly higher in Cluster 1 compared with Cluster 2 at 30 min, paralleling the higher plasma glucose concentrations (Supplemental Figs. S4). Conversely, at 60 min, insulin concentrations became significantly higher in Cluster 3 than in Cluster 2. At later time points, Cluster 3 showed lower insulin concentrations than Clusters 2 and 1 at 180 min, and than Cluster 1 at 240 min.

The more efficient early insulin response in Cluster 3 compared with the other two clusters was further confirmed by a shorter average time to the insulin peak after the start of the meal (66.67 ± 20.00 min), compared with Cluster 1 (110.77 ± 22.53 min) and Cluster 2 (115.56 ± 40.51 min) (all $p < 0.05$).

3.4. Association between clusters of CGM-defined postprandial glucose response and parameters of insulin secretion/function

In the subgroup of 50 participants with plasma glucose and insulin measurements after the standardized meal consumed at the clinic, indices of insulin secretion and sensitivity were estimated over the 4-h postprandial period.

The results for ISSI-2 and Matsuda index were consistent with those obtained from the HOMA indices based on fasting measurements. Specifically, Cluster 1 showed the lowest insulin secretion (ISSI-2) and sensitivity (Matsuda), Cluster 3 the highest ones, and Cluster 2 exhibited intermediate values. However, as reported in Table 3, statistically significant differences were observed only between Cluster 1 and the other

Table 3
Associations between clusters of CGM-defined postprandial glucose response and measurements of insulin secretion and action.

	Cluster 1 $N = 13$	Cluster 2 $N = 27$	Cluster 3 $N = 10$
Insulin secretion-sensitivity index-2 (ISSI-2)	0.70 ± 0.13	0.99 ± 0.36*	1.27 ± 0.36*
Matsuda index	1.82 ± 0.62	2.74 ± 1.23*	3.07 ± 0.68*
Disposition index	1.02 ± 0.67	2.08 ± 1.47*	2.37 ± 1.05*
60-min Insulinogenic index	0.84 ± 0.31	0.84 ± 0.58	1.67 ± 1.07*†
$iAUC_{0–120min}$ Insulin/Glucose	0.98 ± 0.29	1.18 ± 0.66	2.08 ± 1.18*†

Legend: *p < 0.05 vs. Cluster 1; † p < 0.05 vs. Cluster 2 according to Bonferroni post-hoc test. All reported indices of insulin secretion and sensitivity are dimensionless.

two clusters. These findings were largely confirmed after adjustment by covariance analysis for both metformin use and HbA1c levels, with ISSI-2 remaining significantly lower in Cluster 1 than in Cluster 3 ($p = 0.038$), but not versus Cluster 2 ($p = 0.115$).

Focusing on indices assessing the postprandial insulin response at the earlier time points, individuals in Cluster 3 showed a better early insulin response compared with those in the other two clusters. In particular, participants in Cluster 3 exhibited significantly higher 60-min post-meal insulinogenic index values (1.67 ± 1.07) than those in Cluster 1 (0.84 ± 0.31) and Cluster 2 (0.84 ± 0.58) (all $p < 0.05$). This was consistent with the higher insulin-to-glucose $iAUC_{0–120min}$ ratio observed in Cluster 3 relative to Cluster 1 and 2. Adjustment for both metformin use and HbA1c levels did not alter the observed differences in the insulinogenic index and insulin-to-glucose $iAUC_{0–120min}$ ratio between Cluster 3 and 1 ($p = 0.004$ and $p = 0.002$, respectively), nor between Cluster 3 and 2 (p = 0.002 and p = 0.002, respectively).

4. Discussion

We identified three distinct clusters of glucose response to a standardized meal in a sample of individuals with T2D in good glycemic control as assessed by HbA1c, based on CGM-derived parameters of the postprandial glucose curve. This is the first study quantifying dynamic aspects of the glucose response to a meal consumed at home, beyond iAUC or 2-h postprandial glucose. Clusters were independent of age, diabetes duration or anthropometric measures, while insulin secretion and sensitivity assessed during fasting, progressively improved from Cluster 1 to Cluster 3, with Cluster 2 showing an intermediate profile. However, when we evaluated insulin sensitivity and the plasma insulin response following a standardized meal in a subgroup of participants, distinct features of the three clusters emerged: Cluster 1 showed clear impairments in both insulin sensitivity and early-phase insulin secretion; Cluster 2 was primarily characterized by impaired early-phase insulin secretion, while insulin sensitivity was not markedly altered; and Cluster 3 exhibited relatively preserved insulin sensitivity and early-phase insulin secretion. In general, all markers of insulin sensitivity and early postprandial insulin response were inversely associated with the dynamics of the glucose response. In particular, higher early insulin secretion distinguished Cluster 3, characterized by a rapid return of postprandial glucose to fasting levels, from the other two clusters with a more prolonged postprandial glucose elevation. Nevertheless, the higher rate of postprandial glucose rise observed in Cluster 3 contrasts with its higher early insulin secretion and may be explained by the lower metformin use among individuals in this Cluster. Indeed, metformin is well known to reduce hepatic glucose production after a meal [21].

The hypothesis that the shape of the postprandial glucose curve could help identify underlying pathophysiological impairments, has been previously explored using OGTT in individuals without diabetes [8,22]. This approach was successful in demonstrating that people with distinct glucose response patterns differed regarding the acute insulin response and insulin sensitivity. However, assessing PPGR by an OGTT does not take into account the interplay between carbohydrates and other nutrients in modulating the PPGR and the pathophysiological mechanisms involved in its regulation. To the best of our knowledge, this is the first study in T2D examining the inter-individual variability of glucose response patterns to a standardized mixed meal monitored for 4 h with CGM under real-life conditions.

A key strength of the present study is using multiple curve-derived parameters rather than relying solely on iAUC. While widely applied in studies assessing PPGR [6,7], iAUC reduces the postprandial curve to a single value and, therefore, cannot distinguish between qualitatively different response shapes—e.g., rapid versus delayed peaks, or prolonged returns to baseline. In contrast, our feature set captures temporal and kinetic aspects of the glucose trajectory (e.g., peak timing, slopes, recovery). These parameters better reflect underlying pathophysiological processes, offering a richer basis for identifying meaningful subtypes of T2D. Indeed, while our clustering approach based exclusively on dynamic PPGR parameters does not incorporate baseline clinical and metabolic characteristics—such as age, adiposity, insulin resistance, or glycaemic status—used in alternative clinical subtyping frameworks [23], we prioritized PPGR shape to directly uncover pathophysiological mechanisms underlying T2D to be targeted with precision therapeutic strategies.

This approach further proved to be informative when we adjusted comparisons of postprandial insulin secretion and sensitivity indices for HbA1c levels. Indeed, insulin sensitivity remained significantly lower in Cluster 1 than in the other two clusters (as indicated by the Matsuda index), while early insulin secretion was significantly higher in Cluster 3 compared with the other two clusters. Conversely, fasting-derived indices of insulin secretion and function (i.e., HOMA2-B and HOMA-IR) were no longer significantly associated with clusters after adjustment for HbA1c, suggesting that these measures are more closely related to overall glycemic control. Taken together, these findings support that the identified clusters capture information beyond that provided by fasting or average measures of glucose-insulin regulation. Importantly, the observed differences in the pathophysiological mechanisms regulating PPGR across clusters persisted after adjusting for metformin use, indicating that they are not primarily driven by differences in treatment background.

Another strength of our study is that PPGR parameters are derivable using automated formulas, similar in simplicity to indices of insulin secretion or sensitivity. This avoids subjective manual annotation and enhances reproducibility across studies. The feature extraction pipeline can be applied directly to raw CGM measurements with minimal preprocessing, making it scalable and suitable for epidemiological cohorts or clinical settings where standardization is essential. Furthermore, compared with plasma glucose analysis after the standardized meal, CGM—with its higher resolution—provides a more accurate reflection of an individual's dynamic curve, capturing critical information that may be lost with the more invasive and cumbersome venous sampling.

Our study also has some weaknesses that should be acknowledged. Compared with prior work reporting modest reproducibility or accuracy [24,25], our findings align with the broader challenge of defining stable subgroups of individuals based on the PPGR [26]. Indeed, we found that the cluster assignments derived from a single standardized meal showed only moderate consistency. However, additional analyses demonstrated that the overall glucose response curve was reproducible across repeated meals, with moderate to good agreement throughout the 4-h period. Moreover, comparisons between postprandial glucose concentrations measured by CGM and those obtained from plasma samples at selected time points, showed consistently moderate to good agreement. These findings suggest that the moderate stability of cluster assignment is not primarily driven by CGM measurement noise, but rather reflects the inherent biological variability in postprandial glucose regulation. As with any unsupervised learning approach, resulting clusters depend on feature scaling, distance metric, and cluster number. Although we used established validation indices (Silhouette, Davies-Bouldin) to guide

selection, alternative algorithms or parameterizations could yield slightly different clusters. This methodological sensitivity should be considered when interpreting the biological significance of the identified subtypes.

From a clinical perspective, these considerations imply that cluster assignment – particularly when derived from a single meal test – should be interpreted with caution, possibly considering factors that contribute to day-to-day glucose variability (i.e., physical activity, stress, psychological status, sleep, menstrual cycle, etc.). Nevertheless, it provides valuable insight into postprandial glucose metabolism that should be integrated with complementary measures of glycemic control and the underlying mechanisms of glucose homeostasis to support a personalized dietary approach. Since averaging repeated measurements substantially improved the reproducibility of CGM-derived postprandial glucose parameters (ICC up to ~0.8), the use of repeated standardized meal assessments to enhance classification stability may be particularly relevant in interventional or precision nutrition research settings, where capturing an individual's dominant response pattern may be more informative for detecting temporal changes or predicting clinical outcomes than relying on a single measurement.

An intrinsic limitation of our study, focused on individuals with well-controlled T2D, is that it could not capture a broader spectrum of T2D subtypes, particularly those characterized by advanced β-cell disfunction or markedly reduced insulin secretion capacity. However, we selected patients who are more likely to benefit from improvements in postprandial glucose regulation through appropriate dietary strategies. Additionally, the limited pharmacological interference allowed us to better evaluate the underlying pathophysiological mechanisms driving different PPGR patterns.

As for translational potential of the findings presented in this paper, it is worth noting that the recent ADA Standards of Care [27] highlight the value of CGM in supporting glycemic management and recommend its use in adults with T2D on non-insulin therapies to achieve individualized glycemic targets. Periodic CGM can guide medication and lifestyle adjustments when continuous use is not feasible. This supports implementing more personalized and effective strategies to optimize PPGR and overall glucose profiles in individuals with T2D, using comprehensive CGM data rather than only 2-h post-meal glucose. Within this framework, our study presents a feasible approach to identify distinct PPGR patterns, potentially representing markers of the predominant impairment—beta-cell dysfunction and insulin resistance—in individuals with T2D. However, our methodology requires replication in larger and diverse populations, and the clinical and prognostic value of CGM-derived clusters needs to be established in prospective studies. Future research could leverage this approach to identify markers of disease progression and the risk of long-term diabetic complications. It could also be considered in the context of precision nutrition [28] to target distinct PPGR dynamics, such as reducing early glucose peaks (e.g., low Glycemic Index foods, viscous fiber, higher protein, etc.) or managing prolonged PPGR due to impaired late glucose disposal (e.g., wholegrains, polyphenols, fermentable fiber, etc.).

In conclusion, our study showed that CGM-derived parameters of glucose response to a mixed meal consumed at home can identify distinct PPGR patterns in T2D, reflecting differences in beta-cell function and insulin sensitivity. This approach goes beyond conventional postprandial metrics and supports the potential of CGM-based profiling to inform precision management strategies in T2D.

CRediT authorship contribution statement

Annalisa Giosuè: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Viktor Skantze:** Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis. **Roberta Testa:** Investigation, Data curation. **Giovanna D'Abbronzo:** Investigation, Data curation. **Giuseppina Costabile:** Resources, Investigation. **Marilena Vitale:** Investigation, Data curation. **Alessandra Corrado:** Validation, Methodology, Formal analysis. **Mats Jirstrand:** Validation, Methodology, Formal analysis. **Rikard Landberg:** Writing – review & editing, Supervision. **Gabriele Riccardi:** Writing – review & editing, Supervision, Conceptualization. **Lutgarda Bozzetto:** Supervision, Methodology, Funding acquisition, Conceptualization.

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Declaration of competing interest

All authors declare that there is no duality of interest associated with this manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2026.156626.

Data availability

The data supporting the findings of this study are not publicly available due to sensitivity reasons but are available from the corresponding author upon reasonable request.

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