Differential Responders to a Mixed Meal Tolerance Test Associated with Type 2 Diabetes Risk Factors and Gut Microbiota—Data from the MEDGI-Carb Randomized Controlled Trial

Abstract

- They aimed to fit a simple mathematical model on participants (81 females and 74 males) with elevated T2DM risk, to identify Groups of people who have similar blood sugar responses to the meals. And investigate their association with T2DM risk factors and gut microbiota.

- Results:

Cluster A: People in this group tend to have lower average blood sugar levels (HbA1c), waist circumference, and better insulin sensitivity. (lower blood sugar peaks)

Cluster B: People in this group tend to have higher HbA1c, larger waistlines, and poorer insulin sensitivity. (higher blood sugar peaks)

The clusters correlated with known risk factors for T2DM, such as higher HbA1c levels, larger waist circumference, and lower insulin sensitivity indices.

Why Is This Important?

- Personalized Nutrition: By identifying how different people respond to the same meal, healthcare providers can tailor dietary recommendations to prevent or manage T2DM more effectively.
- Predicting Risk: This method can help predict who is at higher risk for developing T2DM based on their blood sugar response to meals and gut bacteria profile

Introduction

- Major risk factors for the development of T2DM:

  heredity,

  increased waist circumference,

  increased HbA1c

- Elevated glycemic variability and postprandial glucose and insulin responses may affect the risk of developing T2DM and CVD among non-diabetic individuals.

What is the Mixed Meal Tolerance Test (MMTT)?

- The MMTT is used to evaluate the body's ability to regulate blood glucose levels after eating a mixed meal

Reasons for a Mixed Meal:

1. **Realistic Simulation:** A mixed meal is more closely like a typical meal that a person might consume in everyday life.

2. **Comprehensive Evaluation:** Different macronutrients affect glucose metabolism and insulin secretion differently. If we convert the glucose fluctuations into a sine wave, carbohydrates would have higher amplitude peaks compared to proteins and fats, reflecting their greater influence on blood glucose levels.

3. **Beta-Cell Function:** The mixed meal insulin that is secreted from the pancreas is similar to a regular meal. This helps in assessing the functionality of beta cells (the cells in the pancreas that produce insulin) under normal eating conditions.

4. **Insulin Dynamics:** Initial insulin response to carbohydrates, and the prolonged insulin release influenced by proteins and fats.

What is the Oral Glucose Tolerance Test (OGTT)?

- The OGTT is a test that measures the body's ability to process glucose

Key Differences:

1. **Type of Intake**:
   - OGTT: Involves a glucose solution (pure carbohydrate).
   - MMTT: Involves a mixed meal (carbohydrates, proteins, fats).
2. **Measurement Focus**:
   - OGTT: Primarily measures glucose response.
   - MMTT: Measures glucose and other metabolic responses.
3. **Real-World Relevance**:
   - OGTT: Provides information on how the body handles a high glucose load.
   - MMTT: Provides a more realistic assessment of how the body processes a typical meal.

Materials and Methods

The MEDGI-Carb trial was an international multi-center randomized, controlled, parallel-group dietary trial that lasted 15 weeks. It included:

- **3-week baseline period**: Initial period to establish participants' normal dietary and health metrics.
- **12-week controlled dietary intervention**: Period where participants followed specific dietary guidelines.
- 155 participants (81 females and 74 males)

Participant Features:

Feature	Range/Details
Age	18-70 years
BMI (Body Mass Index)	25-40 kg/m^2
Elevated Risk Factors	Prediabetes, family history of diabetes, high waist circumference, elevated fasting glucose, high HbA1c

Intervention Details

Aspect	Details
Diet Style	Mediterranean-style
Caloric Control	Isocaloric, weight-maintenance
Duration	12 weeks
Groups	Low-GI diet, High-GI diet
Digestible Carbohydrates	270 grams per day
Dietary Fiber	35 grams per day
Common Carbohydrate Sources	Half of daily carbohydrate intake (135 grams) from vegetables and fruit identical to each group
Differential Carbohydrate Sources	Other half: Low-GI group: GI < 55. High-GI group: GI > 70
Energy Intake Modulation	Adjusted by varying protein and fat intake
Goal	To evaluate the effects of different GI diets on participants at risk of T2DM

- The study calculated three different insulin sensitivity indices (QUICKI, Stumvoll, and Matsuda) using data from OGTTs.

Index	Description	Calculation & Data Source	Importance
QUICKI	Simple method to estimate insulin sensitivity	Uses fasting blood glucose and insulin levels	Lower values indicate higher insulin resistance
Stumvoll Index	Estimates insulin sensitivity using OGTT data	Uses glucose and insulin levels during OGTT	Dynamic assessment of insulin sensitivity
Matsuda Index	Comprehensive measure of whole-body insulin sensitivity	Uses fasting and post-load glucose and insulin levels from OGTT	Combines fasting and postprandial states

- Blood samples were collected at TP (time point) 15 after the test meal and then at TP 30, TP 45, TP 60, TP 90, TP 120, TP 180, and TP 240.

1. **DNA Extraction**:
   - They took DNA from bacteria in fecal samples (poop).
2. **PCR Amplification**:
   - They copied a specific part of bacterial DNA (16S rRNA gene) that helps identify different bacteria.
3. **Sample Preparation**:
   - Added barcodes to each sample so they could tell them apart later.
   - Added adapters to help with sequencing.
4. **Sequencing**:
   - Sent the prepared samples to a high-tech machine (Illumina NovaSeq 6000) to read the DNA sequences.
5. **Data Analysis**:
   - Used software (QIIME2) to:
     - Separate the DNA sequences by their unique tags.
     - Clean up the data to remove poor-quality sequences.
     - Identify the types of bacteria based on the DNA sequences.
6. **Purpose**:
   - To see if gut microbiota could explain why people have different blood sugar responses after eating.
   - Focused on specific bacteria known to be linked to blood sugar control: Bifidobacterium, Bacteroides, Faecalibacterium, and Akkermansia……..

- They used a simplified glucose model (*proposed by Bolie)* to analyze blood sugar changes after a carbohydrate-rich breakfast, aiming to group people based on their responses and relate these groups to type 2 diabetes risk.

The model assumes a simple linear relationship between insulin and glucose, even though this is an oversimplification.

It treats the meal as an immediate spike in glucose at the first measurement time

Key Equation:

$$G(t) = G_b + A\sin(\omega t)e^{-\alpha t}$$

Where:

- $G(t)$: Blood glucose level at time t.
- G_b: Baseline glucose level.
- A: Amplitude of the glucose peak.
- ω: Frequency of glucose oscillations.
- α: Damping coefficient.

represents the damping effect, showing how quickly the oscillations die down.

represents the oscillatory nature of glucose levels.

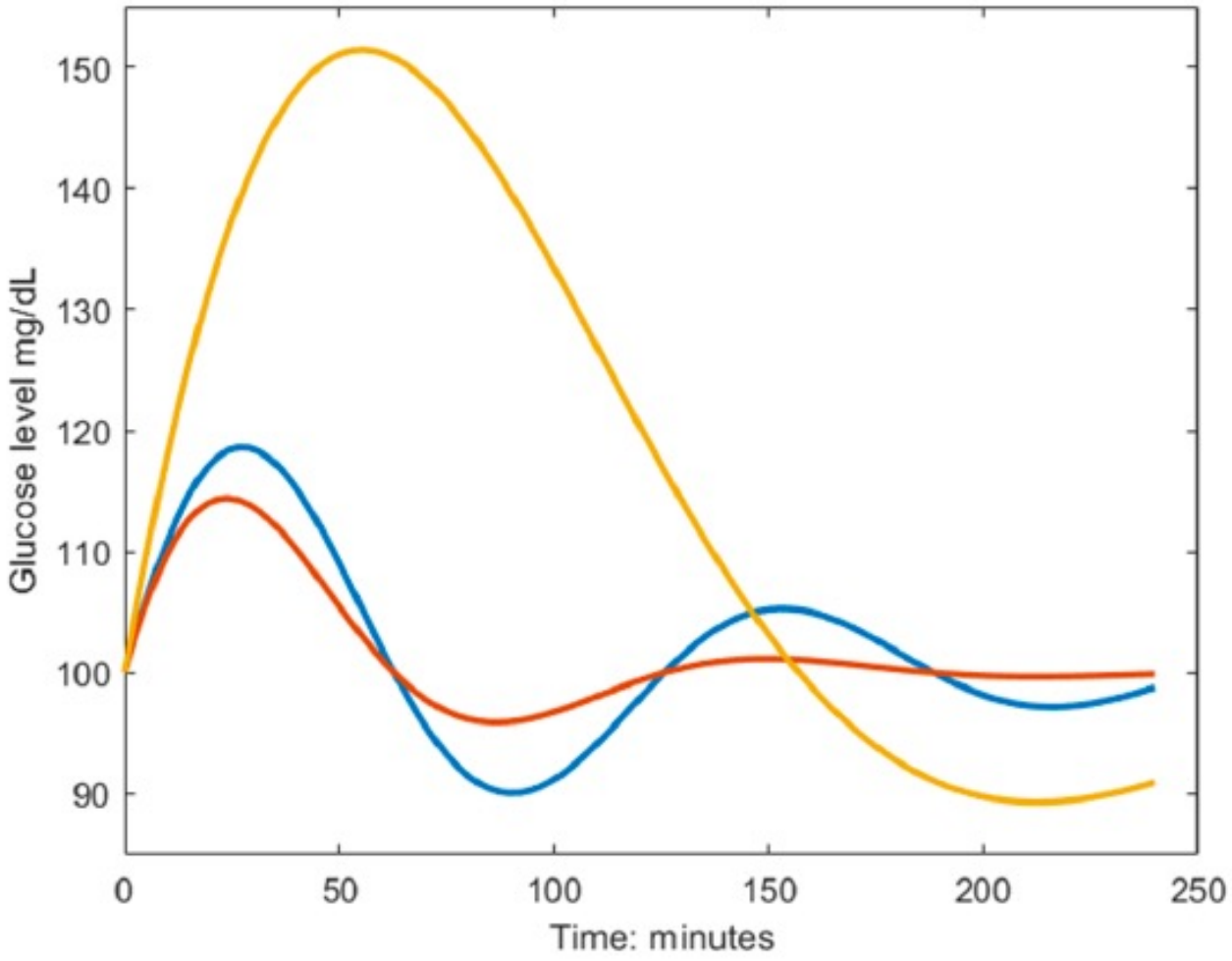
Example

Blue Curve:
- High Frequency
- Low Amplitude

Red Curve:
- **Damping Coefficient (α)**: High
- **Amplitude :** Moderate

faster return to baseline

Yellow Curve:
- **Description**: Slow response with poor glucose regulation.
- **Low Frequency**
- **High Amplitude**



Equation (2) helps calculate the peak blood glucose level after eating a meal

$$G_{\max} = G_b + \frac{e^{-\kappa \cos^{-1}\left(\kappa/\sqrt{1+\kappa^2}\right)}}{\sqrt{1+\kappa^2}} A$$

It adjusts the amplitude of the glucose response based on how quickly glucose levels rise and fall (frequency) and how quickly they return to baseline (damping).

Where:

- $G_{\max}$: Maximum glucose concentration after eating.
- G_b: Baseline glucose level.
- A: Amplitude, indicating the height of the glucose peak.
- κ: A parameter defined as $\kappa = \frac{\alpha}{\omega}$
  - α: Damping coefficient, indicating how quickly glucose levels return to baseline.
  - ω: Frequency, indicating how quickly glucose levels oscillate.

estimated from the data and remains constant for that individual's mode for both equations.

It is static for each individual's model but varies between individuals.

Simplified Breakdown of Equation (2):

1. **Maximum Glucose Calculation**:
    - This equation calculates the peak glucose level ($G_{\max}$) after eating, starting from the baseline level (G_b).
2. **Exponent Term ($e^{-\kappa \cos^{-1}\left(\kappa/\sqrt{1+\kappa^2}\right)}$)**:
    - Adjusts the amplitude based on the damping and frequency parameters.
3. **Denominator Term ($\sqrt{1+\kappa^2}$)**:
    - Normalizes the amplitude adjustment to account for the combined effect of damping and frequency.

Statistical Analyses

Parameters Defined:

$$\phi_i = \{G_{b,(i)}, A_i, \omega_i, \alpha_i\}$$

- $G_{b,(i)}$: Baseline glucose level for person i.
- A_i: Amplitude (height of glucose peak) for person i.
- ω_i: Frequency (how quickly glucose levels oscillate) for person i.
- α_i: Damping coefficient (rate of return to baseline) for person i.

3. Regression to Glucose Measurements:

Equation (3):

$$y_i = G(\phi_i, t) + \epsilon$$

- y_i: Observed glucose measurement for person i.
- $G(\phi_i, t)$: Model prediction based on parameters ϕ_i at time t.
- ϵ: Error term (random variation), assumed to be normally distributed with mean 0 and constant variance.

Equation (4):

$$\phi_i = \beta e^{\eta_i + A x_i}$$

- β: Fixed effects shared among all individuals.
- η_i: Random effects specific to individual i, accounting for variability.
- x_i: Covariates (additional factors) affecting the parameters, with A being a covariate matrix.

What It Means:

- The individual parameters ϕ_i are influenced by both fixed effects (common to all) and random effects (unique to each person).
- Covariates x_i (like age, weight, etc.) also influence the parameters.

Mean Vector

A mean vector represents the average value of the random effects for a group of individuals (a cluster)

How Mean Vector Works:

1. **Mean Vector (μ_j) for Cluster A**:
    - If the mean vector μ_A is $[0.1, 0.05, 0.02]$:
        - This means that, on average, individuals in Cluster A have:
            - Baseline glucose level 0.1 units higher than the fixed effect.
            - Amplitude 0.05 units higher than the fixed effect.
            - Frequency 0.02 units higher than the fixed effect.
2. **Mean Vector (μ_j) for Cluster B**:
    - If the mean vector μ_B is $[-0.1, -0.05, -0.02]$:
        - This means that, on average, individuals in Cluster B have:
            - Baseline glucose level 0.1 units lower than the fixed effect.
            - Amplitude 0.05 units lower than the fixed effect.
            - Frequency 0.02 units lower than the fixed effect.

Key Concepts:

1. **Groups (M_j)**:
    - These are clusters or groups that individuals are assigned to based on their glucose response patterns.
2. **Indicator Function ($I_{i \in M_j}$)**:
    - This function checks if an individual i belongs to a specific group M_j.
    - If the individual belongs to the group, the function is 1 (true); otherwise, it is 0 (false).

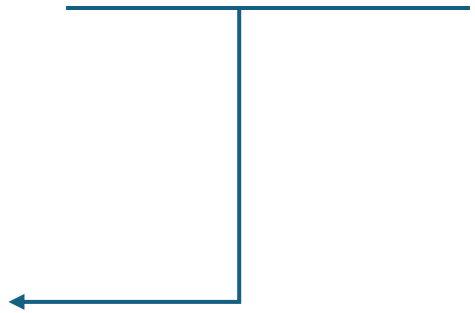
1. **Group Membership and Indicator Function**:
   - Each person i is assigned to a group M_j (e.g., Cluster A or Cluster B).
   - The indicator function $I_{i \in M_j}$ checks which group the person belongs to.
     - If person i is in group M_j, $I_{i \in M_j} = 1$.
     - Otherwise, $I_{i \in M_j} = 0$.
2. **Random Effects as Mixture of Distributions**:
   - The random effects (η_i) for each individual are not drawn from just one normal distribution, but from a mixture of normal distributions.
   - The specific normal distribution used for an individual i depends on the group M_j they belong to.
3. **Multivariate Normal Distributions**:
   - Each group M_j has its own multivariate normal distribution.
   - A multivariate normal distribution considers multiple variables at once (e.g., baseline, amplitude, frequency, and damping), and how they might be correlated.
4. **Mixture of n Multivariate Normal Distributions**:
   - Since there are n groups (e.g., two groups, Cluster A and Cluster B), there are n different multivariate normal distributions.
   - The random effects for an individual i are effectively a mixture of these n distributions, depending on which group they belong to.

- ANOVA is used to check if there are significant differences in continuous measurements (like glucose levels) between groups.
- Chi-squared tests are used to check if there are significant differences in categorical data (like presence or absence of a condition) between groups.

- Researchers compare the abundance of a specific gut bacteria at the start and after 12 weeks.
- They use random forest analysis to identify which species change significantly due to the intervention.
- Cross-validation ensures the results are reliable

$$RSE = 100 \cdot \frac{e_{std}}{\hat{y}}. \tag{5}$$

Here, e_{std} describe the standard error and $\hat{y}$ is the estimate. We consider an RSE value below 50% percent a valid estimate using the Monolix software.



A specialized software used to estimate the model parameters, accounting for both fixed and random effects.

Results

- The parameters were successfully estimated with RSE < 43% in all cases, which indicated certainty in the estimates.

- The damping parameter could not be estimated with enough certainty to be included in the clustering analysis

- The covariate for group membership (whether the individual had a high-GI or low-GI meal) could not be estimated with enough precision. This means there was no significant difference in glucose response between the high-GI and low-GI meals

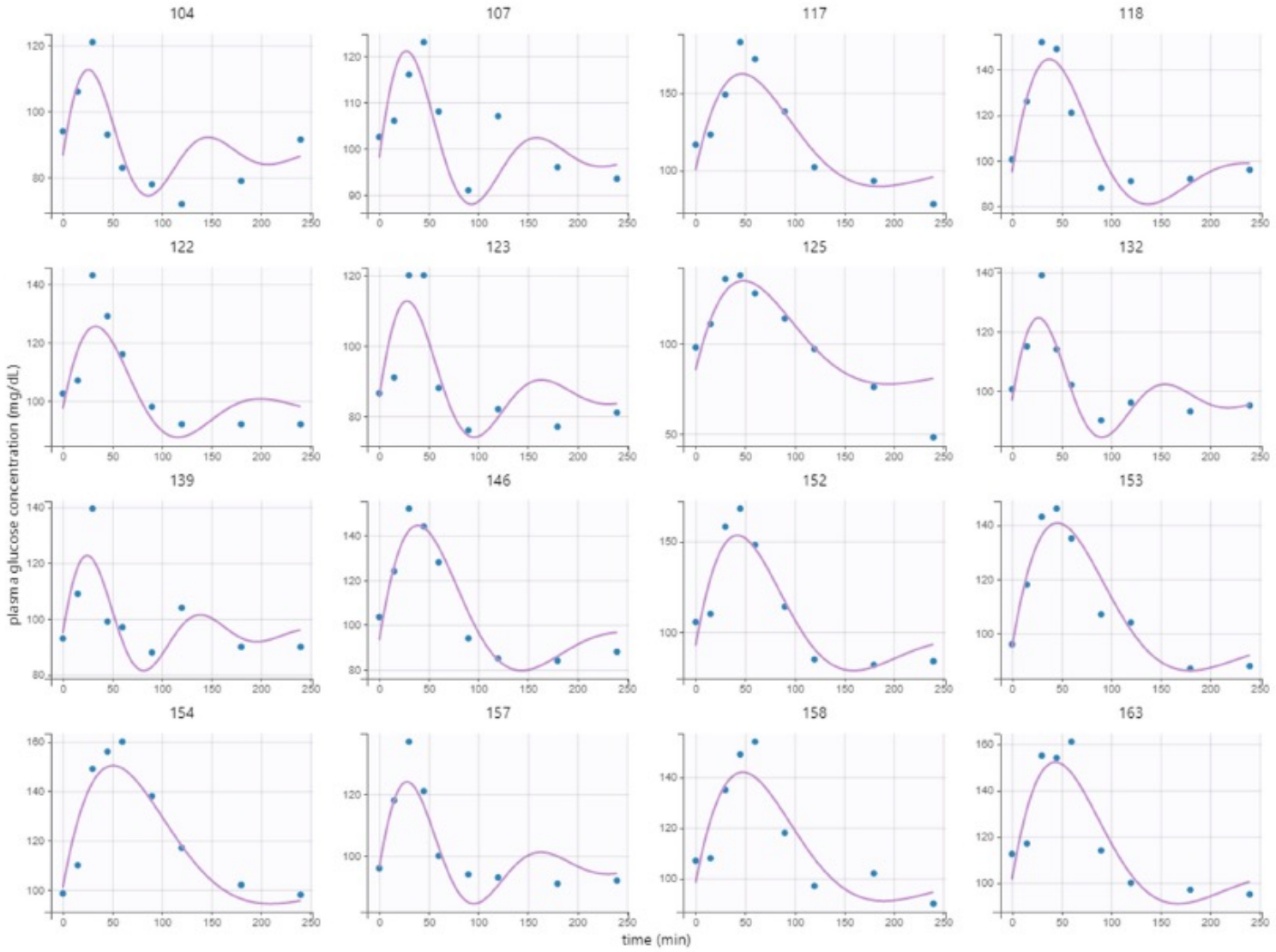
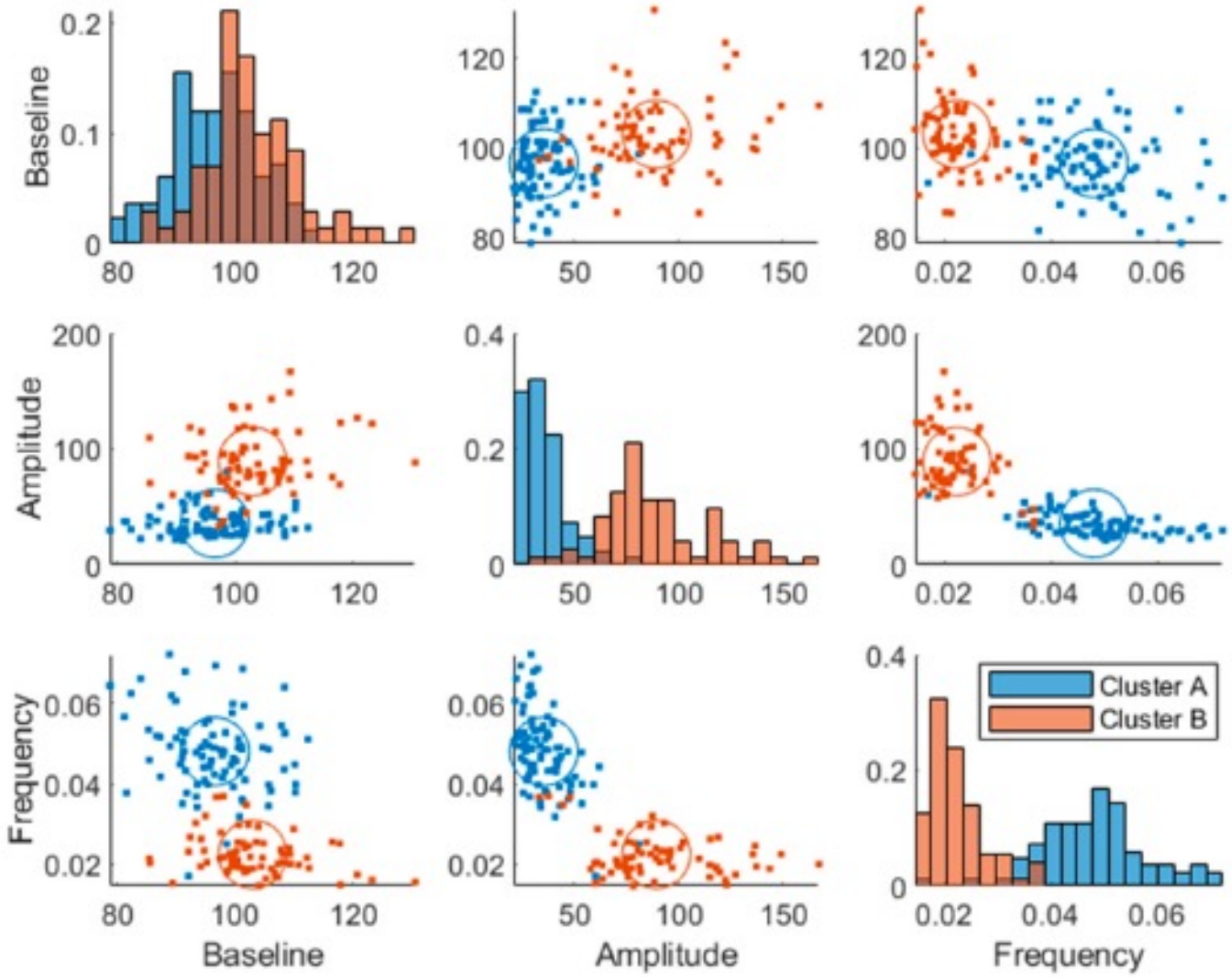


Figure 2. Model fit to postprandial breakfast MMTT response at baseline of 16 randomly selected rep-

Points: These represent the actual glucose measurements taken from the subjects after they ate breakfast.

Lines: These represent the glucose levels predicted by the model using the parameters estimated for each subject

Shows the data at baseline (before the intervention).



These groups were clearly different in terms of how high their glucose levels spiked and how quickly they changed, but not in their starting glucose levels.

The individuals in cluster A had a higher frequency and a lower amplitude.

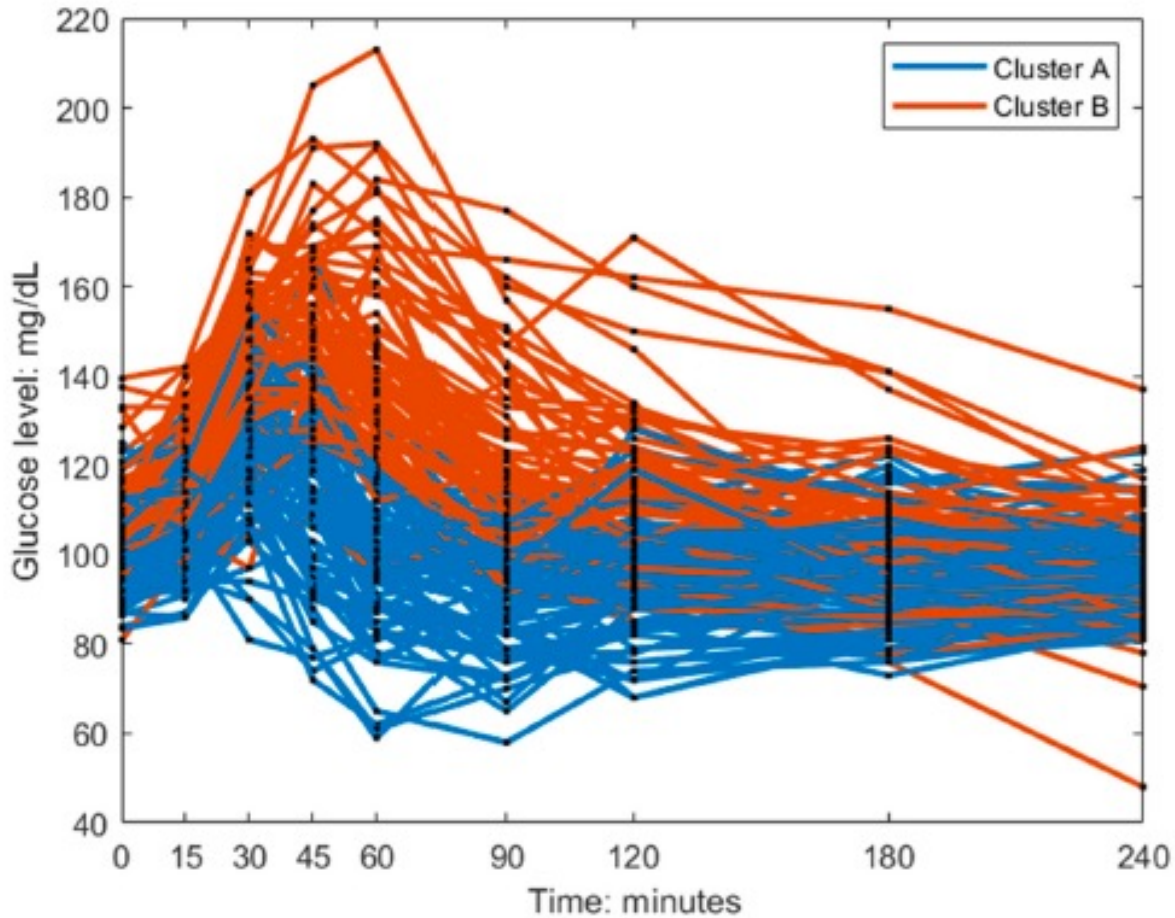


Figure 4. Baseline postprandial breakfast MMTT response color-coded by the clusters.

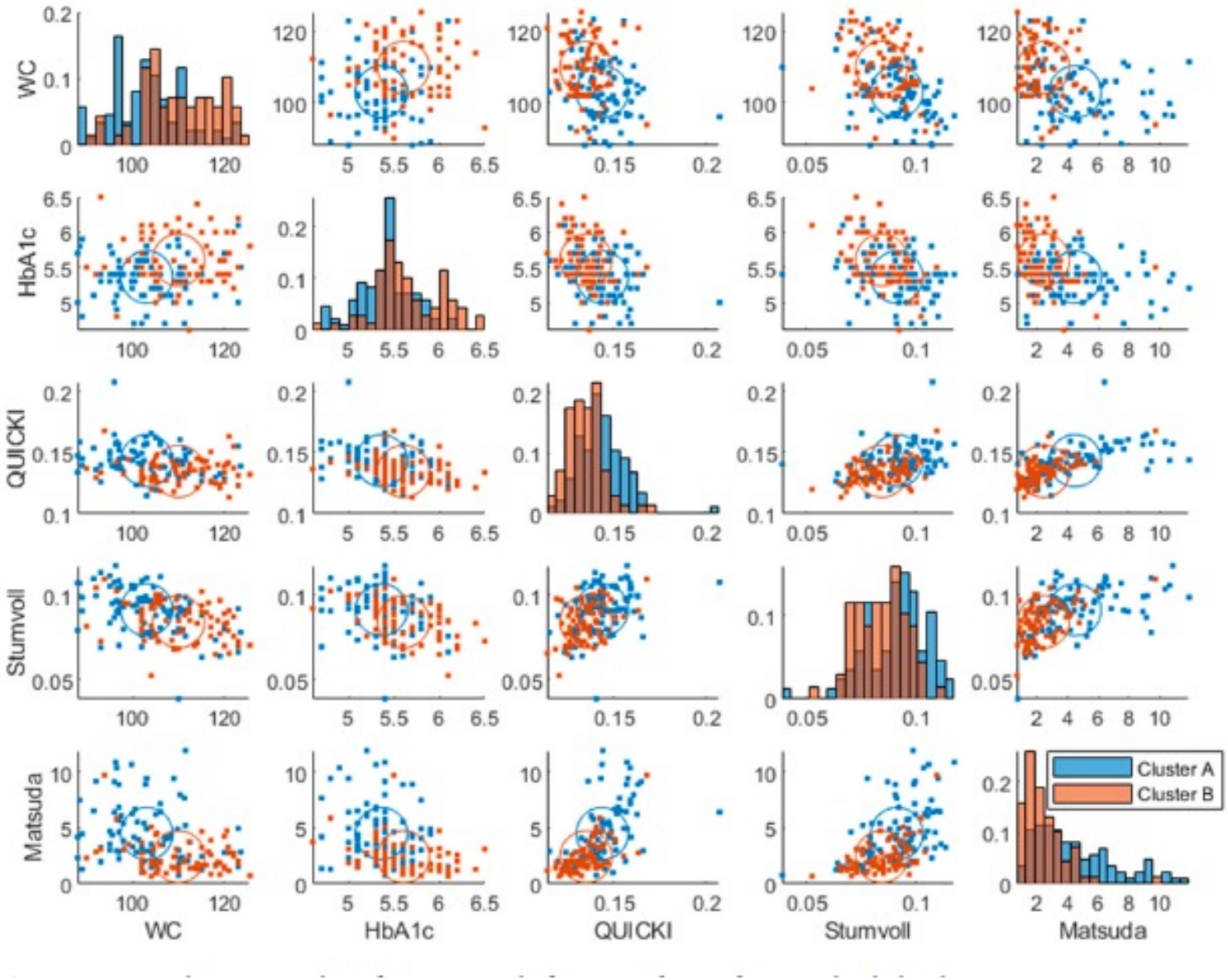


Figure 5 shows the distributions and relationships between different metabolic and glucose control measures for two clusters of individuals

Dots: Normoglycemic (normal glucose regulation)
Triangles: Impaired glucose control
Asterisks: Diabetic

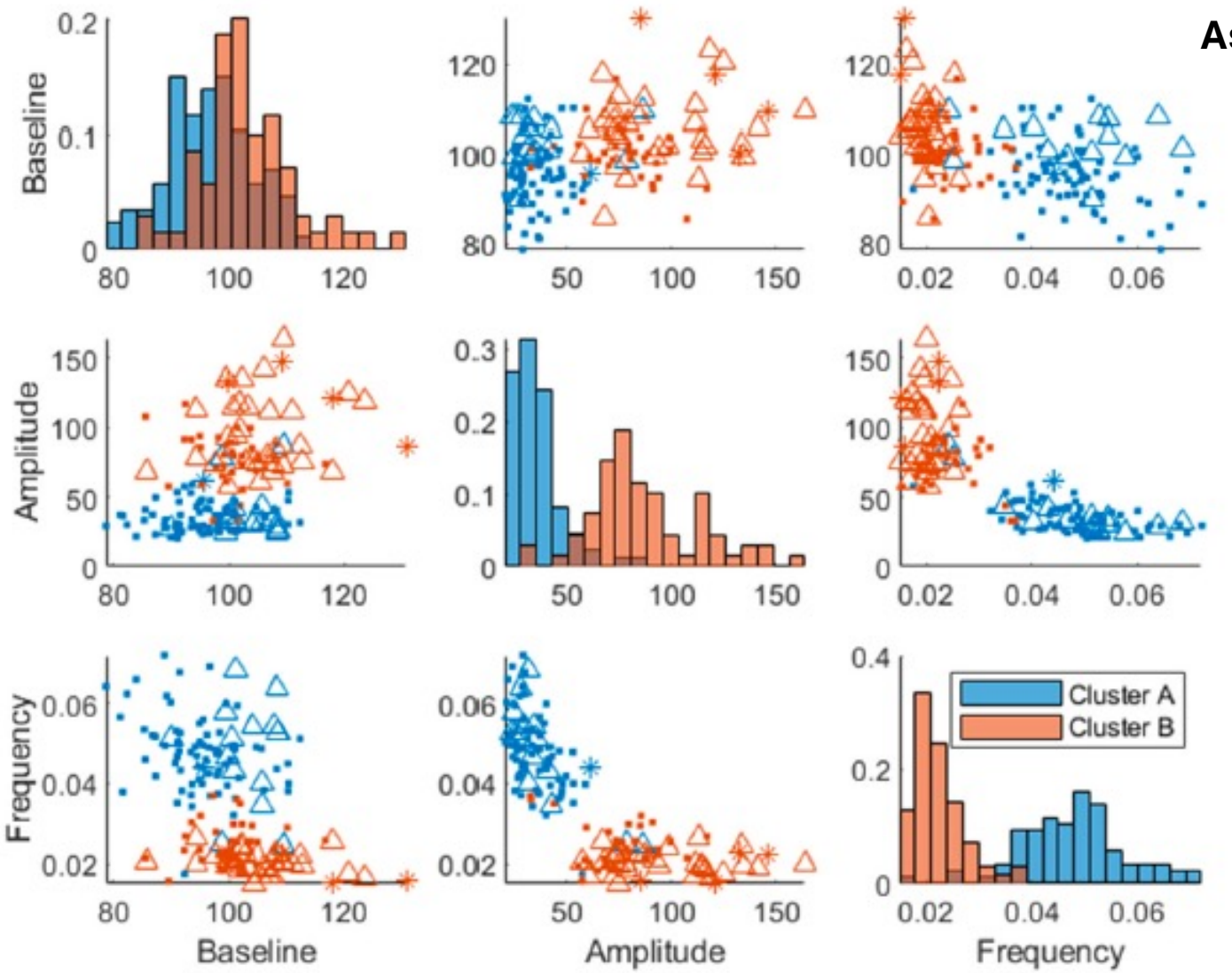
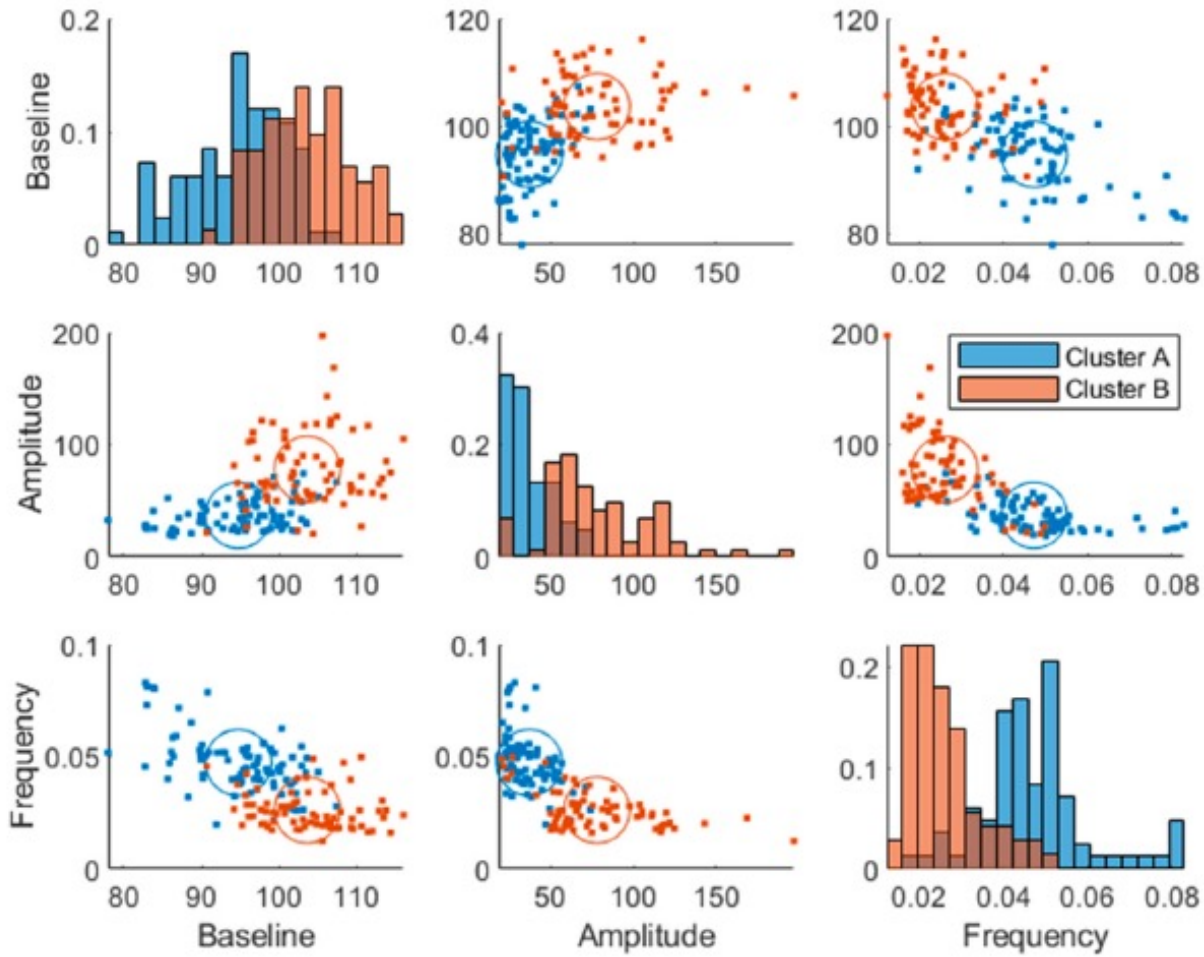


Figure 6 illustrates the distributions and relationships between baseline glucose levels, amplitude, and frequency for two clusters of individuals, highlighting how these parameters vary with glucose regulation status



Shows the data after 12 weeks of intervention

- Thank you