Mixed-meal tolerance test to assess residual beta-cell secretion: Beyond the area-under-curve of plasma C-peptide concentration

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Aims: Residual beta-cell secretion in type 1 diabetes is commonly assessed by area-under-curve of plasma C-peptide concentration (AUC\textsubscript{Cpep}) following mixed-meal tolerance test (MMTT). We aimed to investigate alternative measures of beta-cell responsiveness.

Methods: We analyzed data from 32 youth (age 7 to 17 years) undergoing MMTT within 6 months of type 1 diabetes diagnosis. We related AUC\textsubscript{Cpep} with (a) validated mechanistic index of postprandial beta-cell responsiveness M\textsubscript{I} accounting for glucose level during MMTT, and (b) pragmatic marker calculated as baseline plasma C-peptide concentration corrected for baseline plasma glucose concentration.

Results: Postprandial responsiveness M\textsubscript{I} was correlated with age and BMI SDS (R\textsubscript{s} = 0.66 and 0.44, P < 0.01 and P < 0.05) and was more correlated with glycated hemoglobin than AUC\textsubscript{Cpep} (R\textsubscript{s} = 0.79, P = 0.04). The pragmatic marker was highly correlated with AUC\textsubscript{Cpep} (R\textsubscript{s} = 0.94, P < 0.01).

Conclusions: Postprandial responsiveness M\textsubscript{I} may be more relevant to glucose control than AUC\textsubscript{Cpep}. Baseline C-peptide corrected for baseline glucose appears to be a suitable surrogate of AUC\textsubscript{Cpep} if MMTT is not performed.

KEYWORDS
beta-cell secretion, C-peptide, mixed-meal tolerance test, type 1 diabetes

1 | INTRODUCTION

The area-under-curve of sequential C-peptide concentrations (AUC\textsubscript{Cpep}) during the mixed-meal tolerance test (MMTT) is the gold-standard method to assess residual beta-cell (ie, insulin) secretion in type 1 diabetes.\textsuperscript{1} Traditionally, glucose excursions during the MMTT are not taken into account, although these impact on the magnitude of C-peptide response.\textsuperscript{2}

In this work, we re-analyzed MMTT data obtained in newly diagnosed children and adolescents with type 1 diabetes aged 7 to
17 years\(^2\) (a) to identify surrogate mechanistic and pragmatic markers of AUC\(_{\text{C-pep}}\) and (b) to explore the relationships among demographic and clinical factors, and AUC\(_{\text{C-pep}}\) and its surrogate markers.

## METHODS

We analyzed data obtained from 32 participants with newly diagnosed type 1 diabetes (age 12.4\(^{±}\)2.9 years, 12 males, HbA1c 6.8\(^{±}\)1.1, BMI SDS 0.62\(^{±}\)0.02), total daily dose of insulin 0.57\(^{±}\)0.23 U/kg; mean [SD]) who underwent MMTT within 6 months of diagnosis (mean time since diagnosis 142\(^{±}\)38 days).\(^2\) The National Research Ethics Committee East of England-Cambridge South approved the study.

All participants (aged ≥16 years) gave informed consent, and children <16 years gave assent and their parents gave informed consent to the study procedures.

The MMTT was performed following an overnight fast, with no food or drink other than water from midnight, and at baseline glucose levels between 4 and 11.1 mmol/L. Long-acting insulin and basal rates for insulin pump users were continued as normal. The use of rapid-acting insulin bolus was acceptable up to 2 hours before the MMTT and the use of short-acting insulin bolus up to 6 hours before the MMTT. Participants ingested 6 mL/kg of Boost meal solution (maximum 360 mL), within 10 minutes. Blood samples for the measurement of C-peptide and glucose were collected 10 minutes prior to the meal (–10 minutes), at the time of ingestion (0 minutes), and at 15, 30, 60, 90 and 120 minutes.

Plasma C-peptide was assayed in singleton on a Diasorin Liaison XL automated immunoassay analyzer using a one-step chemiluminescence immunoassay (Diasorin S.p.A, 13040 Saluggia [VC], Italy). Glucose levels were analyzed via an adaption of the hexokinase-glucose-6-phosphate dehydrogenase method.\(^3\) HbA1c was analyzed on the XL automated immunoassay analyzer using a one-step chemiluminescence immunoassay.

The total daily dose of insulin was inversely correlated with MMTT. Participants ingested 6 mL/kg of Boost meal solution (maximum 360 mL), within 10 minutes. Blood samples for the measurement of C-peptide and glucose were collected 10 minutes prior to the meal (–10 minutes), at the time of ingestion (0 minutes), and at 15, 30, 60, 90 and 120 minutes.

## RESULTS

The postprandial and fasting beta-cell responsiveness \(M_1\) and \(M_2\) were estimated at 3.3\(^{±}\)1.6-5.4\(^{±}\) and at 3.1\(^{±}\)2.0-4.5\(^{±}\) 10\(^{-7}\)/minutes, respectively. Figure S1 shows a sample model fit to measured plasma C-peptide including measurements of plasma glucose (the forcing function). Figure S2 depicts weighted residuals across all participants demonstrating acceptable fit of the model to plasma C-peptide measurements.

Table 1 reports the Spearman rank correlation among demographic and clinical factors, AUC\(_{\text{C-pep}}\) and its surrogate markers. The strongest correlations found for age and BMI SDS were with \(M_1\) (\(R_S = 0.66\) and 0.44, respectively, \(P < 0.01\) and \(P < 0.05\), respectively). The total daily dose of insulin was inversely correlated with \(M_0\) (\(R_S = −0.42\), \(P < 0.05\)) and baseline C-peptide over baseline glucose (\(R_S = −0.38\), \(P < 0.05\)).

Figure 1 demonstrates that AUC\(_{\text{C-pep}}\) has a stronger correlation with baseline C-peptide corrected for baseline glucose (\(R_S = 0.94\)) than baseline C-peptide per se (\(R_S = 0.88\)). Figure S3 relates baseline HbA1c vs AUC\(_{\text{C-pep}}\) and log-transformed \(M_1\). AUC\(_{\text{C-pep}}\) was not correlated with HbA1c (\(R_S = −0.19\), \(P = \text{NS}\)) whereas \(M_1\) is (\(R_S = −0.36\), \(P < 0.05\)); the difference between the two correlation coefficients is statistically significant (\(P = 0.04\)).

## DISCUSSION

The present analysis demonstrates the feasibility of using a model of C-peptide kinetics to assess residual beta-cell function during MMTT in newly-diagnosed type 1 diabetes. Traditionally, the AUC\(_{\text{C-pep}}\) during a MMTT has not been corrected for glucose excursions, which are likely to affect the amplitude of the C-peptide response.\(^2\) Our data show that using more advanced measures of beta-cell function, such as \(M_1\) and \(M_2\), can identify meaningful correlations with clinical parameters such as TDD and HbA1c, which are not identified using uncorrected AUC\(_{\text{C-pep}}\). In addition, we show baseline C-peptide corrected for baseline glucose to be a surrogate marker of AUC\(_{\text{C-pep}}\).

The basal responsiveness \(M_0\) and the postprandial responsiveness \(M_1\) were estimated at median 3.3 and median 3.1 10\(^{-7}\)/min, respectively. These values are considerably smaller than those estimated in normal subjects where \(M_0\) were estimated at a mean of 10.3 and \(M_1\) at 9.00 10\(^{-7}\)/min.\(^7\) In two subjects, \(M_1\) was estimated at zero and in one subject \(M_1\) to zero due to the lack of increased C-peptide levels post-meal and undetectable C-peptide level at baseline. These estimations are clinically meaningful as individuals with complete basal and post-prandial insulin responsiveness can be identified.
The positive correlations between age and \( \text{MI} \), and between BMI SDS and \( \text{MI} \) suggest that postprandial responsiveness is more preserved in older and heavier children and adolescents with newly-diagnosed type 1 diabetes than the younger and lighter individuals.

Figure 1 demonstrates that baseline C-peptide corrected for baseline glucose is highly correlated with AUCCpep and could be used as a surrogate marker of insulin secretion instead of AUCCpep. A previous study has shown the plausibility of using 90-min-stimulated C-peptide concentration or baseline C-peptide as a substitute for AUCCpep to represent insulin secretion with a similar correlation coefficient \( R^2 = 0.96 \) but in a larger population \( (N = 421) \). Data from the present analysis suggest that baseline C-peptide corrected for baseline glucose may be a more appropriate marker than baseline C-peptide and a more cost-effective marker than the stimulated C-peptide concentration side-stepping the need for MMTT and complexity of the assessment.

We show that \( M_0 \) was more tightly correlated with HbA1c than AUCCpep \( (P = 0.04) \) indicating that \( M_0 \) may be a more clinically relevant marker of C-peptide secretion than AUCCpep. The study is limited by a relatively small sample size. Further analyses with larger datasets and longitudinal evaluations are warranted. We applied parameters of C-peptide kinetics determined in healthy subjects. As C-peptide is eliminated primarily by the kidney and assuming comparable kidney function among healthy individuals and those with recently diagnosed type 1 diabetes, we consider this limitation to be of little significance to our findings.

Alternative C-peptide secretion models assume a more complex relationship between glucose concentration and insulin secretion compared to the model used in the present study. These alternative models may provide additional information about C-peptide secretory characteristics but require more frequent sampling. Our

### TABLE 1

Spearman rank correlation between demographic/clinical factors and markers of beta-cell responsiveness \( (N = 32) \)

|                       | Age (y) | HbA1c (%) | BMI SDS | TDD (U/kg) | \( \text{AUCC}_{\text{Cpep}} \) (pmol/L/min) | Baseline C-peptide (pmol/L) | Baseline C-peptide over baseline glucose | \( M_0 \) (/min) | \( M_1 \) (/min) | IDAA1c | \( \text{IAUCC}_{\text{Cpep}} \) (pmol/L/min) |
|-----------------------|---------|-----------|---------|------------|--------------------------------|-----------------------------|----------------------------------------|----------------|----------------|--------|---------------------------------|
| Age (y)               | 1.00    | −0.34     | 0.14    | −0.08      | 0.46**                          | 0.27                        | 0.31                                    | 0.27           | 0.66**                      | −0.31  | 0.50                            |
| HbA1c (%)             | 1.00    | 0.01      | −0.01   | −0.19       | −0.08                           | −0.13                       | −0.15                                   | −0.36*         | 0.72**                      | −0.14  | −0.14                           |
| BMI SDS               | 1.00    | 0.41*     | 0.38*   | 0.41*       | 0.36*                           | 0.44*                       | −0.18                                   | 0.69**         |                            |       |                                 |
| TDD (U/kg)            | 1.00    | −0.32     | −0.27   | −0.38*      | −0.42*                          | −0.28                       | 0.64**                                  | −0.22          |                            |       |                                 |
| \( \text{AUCC}_{\text{Cpep}} \) (pmol/L/min) | 1.00    | 0.88**    | 0.94**  | 0.92**      | 0.79**                          | 0.91**                      | −0.36                                   | 0.99**         |                            |       |                                 |
| Baseline C-peptide (pmol/L) | 1.00    | 0.95**    | 0.89**  | 0.54**      | −0.24                           | 0.87**                      |                                 |
| Baseline C-peptide over baseline glucose | 1.00    | 0.63**    | −0.37   | 0.89**      | 1.00                            | 0.79**                      | 0.08                                   | 1.00           |                            |       |                                 |
| \( M_0 \) (/min)      | 1.00    | 0.67**    | −0.35   | 0.91**      |                                 |                             |                                 |
| \( M_1 \) (/min)      | 1.00    | 0.48      | 0.79**  | −0.28       | 1.00                            |                             |                                 |

\*\( P < 0.05 \), **\( P < 0.01 \). Significant correlations are shown in boldface.

Abbreviations: BMI SDS, body mass index SD score; IDAA1c, insulin-dose adjusted HbA1c; \( \text{IAUCC}_{\text{Cpep}} \), incremental area-under-curve of C-peptide; TDD, total daily dose of insulin.

![FIGURE 1](image-url)  
The scatter plot of \( \text{AUCC}_{\text{Cpep}} \) vs baseline C-peptide (A) and \( \text{AUCC}_{\text{Cpep}} \) vs baseline C-peptide corrected for baseline glucose level (B)
parameter $M_t$ represents the dominant relationship between plasma glucose and C-peptide secretion and is accounted for in the alternative approaches.9,10

In conclusion, baseline C-peptide corrected for baseline glucose may be a suitable surrogate marker of residual beta-cell in newly-diagnosed type 1 diabetes. Postprandial pancreatic responsiveness estimated through a model of C-peptide kinetics appears more relevant to glucose control than the conventional area-under-curve of plasma C-peptide concentration following MMTT.

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CONFLICTS OF INTEREST

R.H. reports having received speaker honoraria from Eli Lilly and Novo Nordisk, serving on advisory panel for Eli Lilly and Novo Nordisk, receiving license fees from BBraun and Medtronic; and having served as a consultant to BBraun. M.E.W. has received license fees from Beckton Dickinson and has served as a consultant to Beckton Dickinson. Y.R., M.T., R.H.W. and D.B.D. declare no competing financial interests exist.

AUTHOR CONTRIBUTIONS

Y.R. and R.H. had complete access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Y.R. and R.H. carried out data analysis. Y.R. and R.H. wrote the manuscript. All authors critically reviewed the report.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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