Check for updates

Received: 9 April 2025 | Revised: 30 May 2025 | Accepted: 31 May 2025

DOI: 10.1111/dom.16528

RESEARCH LETTER

WILEY

A simplified protocol based on a standardized meal-stimulated beta-cell response to stratify people living with non-insulin-treated type 2 diabetes

Cathy J. Sun MD[1,2] | Heather Lochnan MD[1,2] | Alexander Sorisky MDCM[1,2] | Julie Shaw PhD[3,4] | Timothy Ramsay PhD[5,6] | Christopher J. Nolan PhD[7,8,9] | Marc Prentki PhD[10,11,12] | Rémi Rabasa-Lhoret MD[12,13]

[1]Division of Endocrinology and Metabolism, Department of Medicine, The Ottawa Hospital, Ottawa, Ontario, Canada

[2]Faculty of Medicine, University of Ottawa; Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

[3]Division of Biochemistry, Department of Pathology and Laboratory Medicine, The Ottawa Hospital, Ottawa, Ontario, Canada

[4]Faculty of Medicine, University of Ottawa, Ottawa, Ontario, Canada

[5]School of Epidemiology and Public Health, University of Ottawa, Ottawa, Ontario, Canada

[6]Methodological and Implementation Research Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

[7]School of Medicine and Psychology, College of Health and Medicine, Australian National University, Acton, Australian Capital Territory, Australia

[8]John Curtin School of Medical Research, College of Health and Medicine, Australian National University, Acton, Australian Capital Territory, Australia

[9]Department of Diabetes and Endocrinology, The Canberra Hospital, Garran, Australian Capital Territory, Australia

[10]Departments of Nutrition, Biochemistry and Molecular Medicine, Université de Montréal, Montreal, Québec, Canada

[11]Montreal Diabetes Research Center, CRCHUM, Montreal, Québec, Canada

[12]Division of Endocrinology and Metabolism, Department of Medicine and Nutrition, Faculty of Medicine, Université de Montréal, Montreal, Québec, Canada

[13]Montreal Clinical Research Institute, Montreal, Québec, Canada

Correspondence
Cathy J. Sun, Division of Endocrinology and Metabolism, Department of Medicine, The Ottawa Hospital, Ottawa, ON, Canada.
Email: csun@toh.ca

Funding information
The Ottawa Hospital Department of Medicine

KEYWORDS: beta-cell response, non-insulin treated, type 2 diabetes

1 | BACKGROUND/CONTEXT

The complex beta-cell and metabolic dysfunction in type 2 diabetes mellitus (T2D) is related to insulin resistance and beta-cell secretory response.[1] Gold standard methodologies to assess beta-cell function are time- and resource-intensive, and reserved for research studies.[2–4] The stimulated beta-cell response reveals beta-cell function that is distinct from the fasting state. The methods based on stimulation by intravenous glucose or arginine are non-physiologic and bypass the incretin response to oral ingestion. The 75-g oral glucose tolerance test does not account for the effects of proteins and fats, which also trigger the incretin response and insulin secretion.[5–7] Mixed meal tolerance tests vary in meal composition between studies and require complicated computer modelling for insulin secretion, kinetics and clearance calculations.[8,9]

Our objective was to determine the feasibility of simplifying the mixed meal tolerance test to a shorter duration, and quantifying the beta-cell response as the area under the curve (AUC) of peripheral blood insulin and C-peptide concentrations. We standardized the stimulus to be a liquid meal replacement drink, which avoids chewing differences that influence the absorption of solid meals,[10] and adds a standardized ingestion completion time. Such a protocol might be

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2025 The Author(s). *Diabetes, Obesity and Metabolism* published by John Wiley & Sons Ltd.

clinically relevant if the inter-individual variability in beta-cell response is high in patients living with T2D to support future stratification potential regarding personalized therapeutic selection such as timely exogenous insulin initiation.

2 | METHODS

This study protocol was approved by the research ethics board (OHSN-REB 20240236-01H) and registered on ClinicalTrials.gov (NCT06462170). The inclusion criteria were T2D, age ≥18 years and not treated with insulin. The exclusion criteria were inability to fast from midnight or allergy to the drink's ingredients. The study participants were recruited from outpatient diabetes clinics.

The study participants fasted from midnight (water allowed), did not take their T2D medications that morning, and arrived between 7 and 8 AM. An intravenous catheter was inserted and baseline blood was drawn for plasma glucose, insulin and C-peptide measurements. The study participants selected one bottle of the vanilla or chocolate flavour of the factory-sealed and ready-to-serve Boost Original meal replacement drink (https://www.madewithnestle.ca/boost/boost-original-chocolate; https://www.madewithnestle.ca/boost/boost-original-vanilla). Boost Original is a widely available meal replacement drink with a history of formulation consistency, and no artificial flavours. The participants drank the entire bottle between time 0 to 5 min, ensuring a standardized ingestion completion time. Each meal replacement drink was 237 mL (230 calories, 34 g carbohydrates, 10 g protein and 6 g fat). Subsequent timed blood draws were at 30, 60, 90 and 120 min. Study participants were given a 500 mL bottle of water to drink between time 5 to 120 min, while they sat upright in comfortable chairs to ensure no physical exertion affected the measurements.

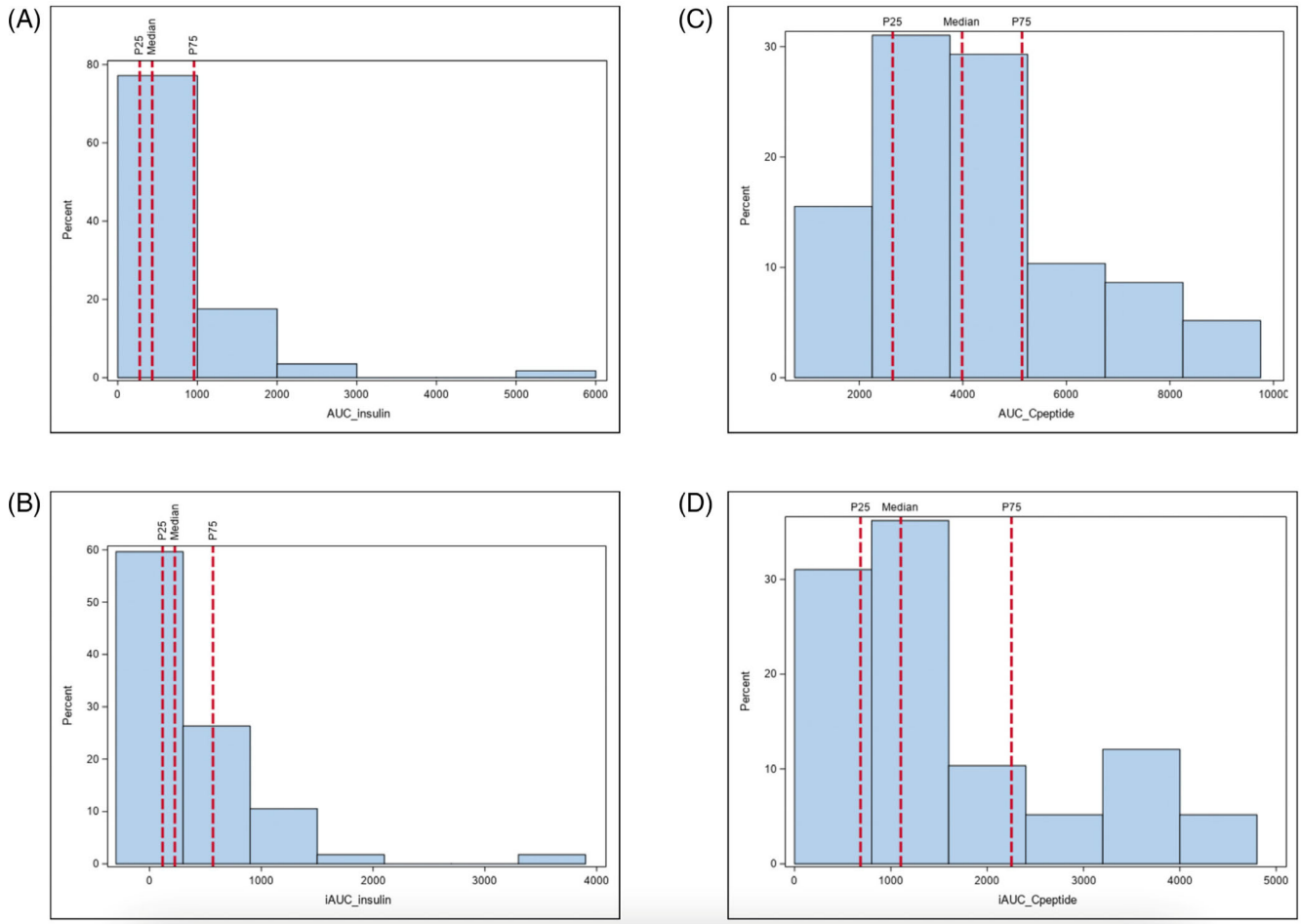

FIGURE 1 Simplified protocol for standardized, meal-stimulated beta-cell response quantification results presented as area under the curve (AUC) and incremental area under the curve (iAUC), which were calculated using the trapezoid rule. The horizontal axes present the $AUC_{insulin}$ and $iAUC_{insulin}$ (pmol/L × hour) (A and B), and $AUC_{C\text{-}peptide}$ and $iAUC_{C\text{-}peptide}$ (pmol/L × hour) (C and D). The AUC and iAUC were calculated for insulin and C-peptide, drawn at times 0, 30, 60, 90 and 120 min. The vertical axis presents the percentage of study participants with AUC and iAUC results which were binned together into histogram bars. The dashed vertical red lines present the 25th percentile (P25), median, and 75th percentile (P75) of the AUC and iAUC results. The coefficients of variation (CV) were the following: (A) $AUC_{insulin}$ CV 1.20 (B) $iAUC_{insulin}$ CV 1.32 (C) $AUC_{C\text{-}peptide}$ CV 0.49 (D) $iAUC_{C\text{-}peptide}$ CV 0.76. For panels (A and B), 57 study participants were included, and 1 study participant was excluded due to hemolysis in insulin samples. For panels (C and D), all 58 study participants were included.

14631326, 2025, 9, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16528 by National Health And Medical Research Council, Wiley Online Library on [29/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

All biochemical measurements were completed at the accredited clinical biochemistry laboratory of the tertiary care hospital on a Roche Cobas 8000 instrument. Plasma glucose was measured by hexokinase assay. Plasma insulin and C-peptide were measured with chemiluminescent sandwich immunoassays.

The AUC and incremental AUC (iAUC) for insulin and C-peptide were calculated using each participant's five timed blood samples (0 to 120 min) to represent the stimulated beta-cell response quantification. The coefficient of variation (CV) for the AUC and iAUC measurements represented the inter-individual variability.

3 | RESULTS

The characteristics of the 58 study participants included: mean age 58.5 (SD 14.5) years; 38% female; mean T2D duration 11 (SD 8) years; HbA1c <7.0%: 38%; HbA1c ≥7.0%: 62%; BMI (kg/m^2) <25.0,

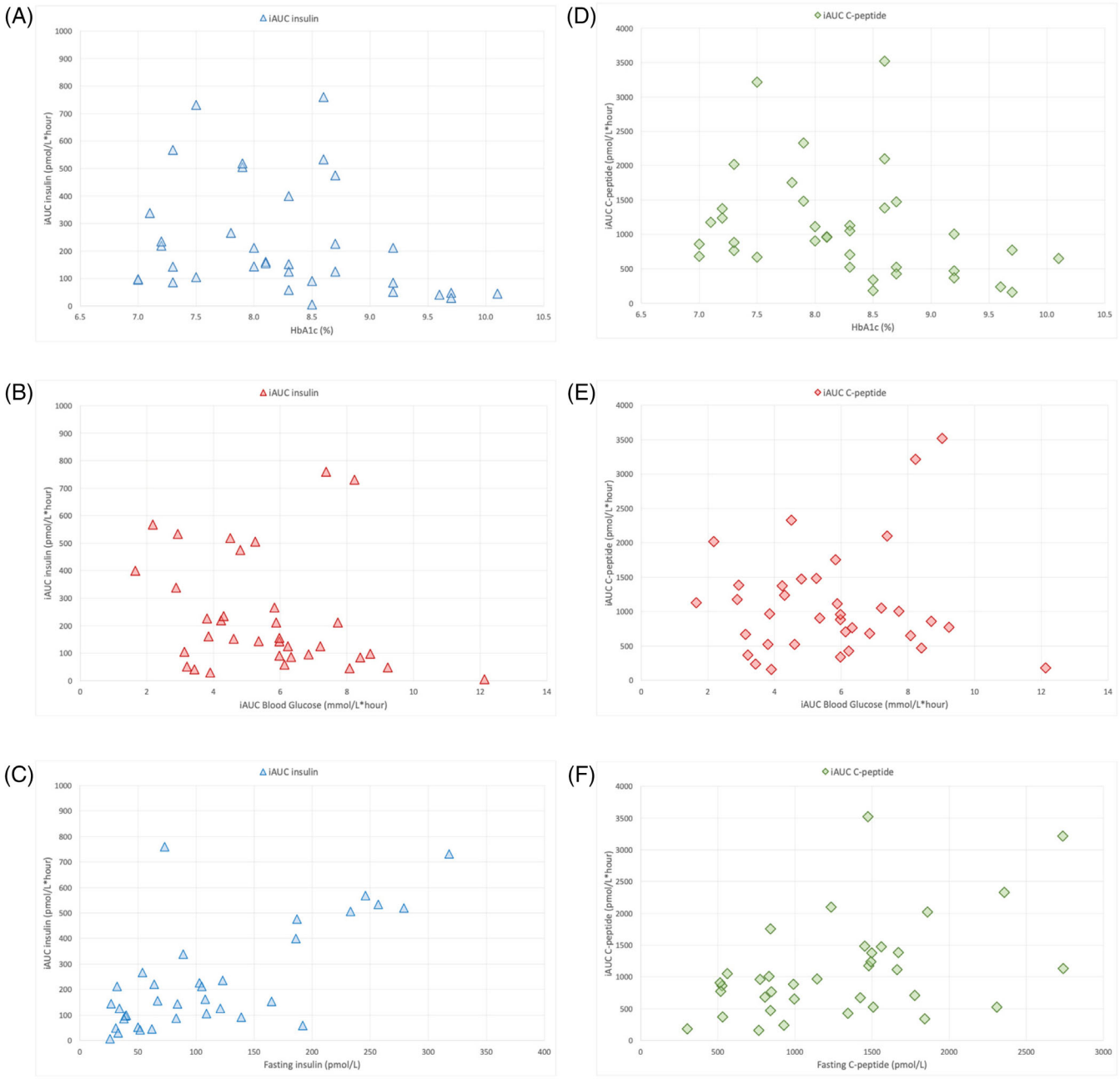

FIGURE 2 Simplified protocol for standardized, meal-stimulated beta-cell response measurements for 36 study participants with HbA1c ≥7.0%. Panels A, B and C scatterplots show that the incremental area under the curve (iAUC) for insulin varies widely over the range of HbA1c (A), iAUC blood glucose (B) and fasting insulin (C). Similarly, panels D, E and F scatterplots show that the iAUC for C-peptide varies widely over the range of HbA1c (D), iAUC blood glucose (E) and fasting C-peptide (F). One study participant was excluded from panels A, B and C because the results were much higher: Fasting insulin was 1098 pmol/L, iAUC insulin was 3781 pmol/L × hour.

14631326, 2025, 9, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16528 by National Health And Medical Research Council, Wiley Online Library on [29/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

25.0–29.9, ≥30.0 for 14%, 38% and 48% of study participants, respectively. The percentage of study participants treated with the following classes of T2D medications were: sulfonylurea 34%, glucagon-like peptide-1 receptor agonist 38%; dipeptidyl peptidase-4 inhibitor 26%, metformin 76% and sodium-glucose cotransporter-2 inhibitor 52%. Patients were on 0 to 4 classes of T2D medications.

Stimulated beta-cell response quantifications are depicted in Figure 1. The $AUC_{insulin}$, $iAUC_{insulin}$, $AUC_{C\text{-}peptide}$ and $iAUC_{C\text{-}peptide}$ results show wide dispersion across the participants, which supports stratification. The higher inter-individual CV for $AUC_{insulin}$ and $iAUC_{insulin}$ than those for C-peptide was expected due to a number of factors, including the high variability of insulin's clearance from the circulation, particularly by the liver, compared to C-peptide.[2–4]

For the 36 study participants with HbA1c ≥7.0%, the scatterplots of this protocol's stimulated beta-cell response $iAUC_{insulin}$ or $iAUC_{C\text{-}peptide}$ and HbA1c or iAUC blood glucose are presented in Figure 2 panels A,B and D,E. This illustrates that this study's stimulated beta-cell response potentially adds new information about each participant's T2D pathophysiologic state, which is not represented by current clinically used glucose-centric parameters (HbA1c, capillary or interstitial glucose levels). Moreover, the fasting insulin and fasting C-peptide only partially reflect the $iAUC_{insulin}$ or $iAUC_{C\text{-}peptide}$ (Figure 2, panels C and F). Therefore, the stimulated beta-cell response allows for more in-depth appreciation of each individual's current T2D pathophysiologic state, which can vary widely from the fasting state measurements. Collectively, Figure 2 suggests that similar levels of HbA1c and iAUC blood glucose may conceal the strikingly different levels of beta-cell response, which are uncovered by stimulation testing of these patients.

4 | DISCUSSION AND CONCLUSIONS

Our simplified protocol for stimulated beta-cell response quantification shows a wide dispersion in the AUC and iAUC for insulin and for C-peptide, indicating that it may be a feasible way to stratify people living with T2D into relatively hyperinsulinemic versus insulinopenic beta-cell response. This may have the potential to add an important new dimension to subtyping T2D and future studies for precision care-based T2D medication selection.[11]

A publication with a similar protocol to ours studied a smaller number of participants ($n = 10$) with T2D <6 years, non-insulin treated, and HbA1c 6.3 (±0.6)% who stopped their T2D medications ≥3 days prior to the test.[12] After consuming a Boost drink that contained more macronutrients than in our study (43 g carbohydrates, 31 g protein and 9 g fat), based on six blood draws over 180 min, their inter-individual variability was lower for $iAUC_{insulin}$ (CV 0.71) and similar for $iAUC_{C\text{-}peptide}$ (CV 0.74). Our study protocol duration is shorter, potentially safer, and more convenient because T2D medications were not held for days before testing.

The limitations of our study include that we quantified peripheral insulin, C-peptide and glucose, which are different from existing insulin secretion research protocols that use complex computerized mathematical modelling to account for their volume of distribution, metabolism, and clearance kinetics.[8,9] C-peptide assays can also vary between manufacturers. This feasibility study was specifically designed to assess the initial performance of our simplified protocol to show wide dispersion reflective of high inter-individual variability in T2D insulin secretion. Therefore, limitations also include lack of comparison to the complex research protocols, and in-depth data analyses given the current small sample size with highly heterogeneous T2D medications.

Precision diabetology is an emerging field, to which our simplified protocol, which requires further studies, could be potentially useful for stratifying T2D subtypes.[11] If this simplified protocol detects insulinopenia, in a suitable clinical context, it could prompt a timely start of insulin pharmacotherapy. Identification of patients with relatively preserved beta-cell response could support refraining from insulin pharmacotherapy and instead prioritizing other management options.[11] Future directions for our simplified protocol for stimulated beta-cell responses include comparison and validation against widely accepted methods, larger studies to enable its optimization (e.g. reducing the duration and number of samples required), and taking into account patient characteristics (e.g. age, anthropometrics, T2D duration, and T2D medication use), glycaemia, liver and kidney function.

AUTHOR CONTRIBUTIONS

Cathy J. Sun: conceptualization, funding acquisition, data curation, resources, methodology, investigations, formal analysis, project administration, supervision, writing – original draft, visualization, writing – review and editing. Heather Lochnan: conceptualization, funding acquisition, writing – review and editing. Alexander Sorisky: conceptualization, methodology, formal analysis, writing – review and editing. Julie Shaw: funding acquisition, resources, writing – review and editing. Timothy Ramsay: conceptualization, funding acquisition, methodology, formal analysis, visualization, writing – review and editing. Christopher J. Nolan: formal analysis, writing – review and editing. Marc Prentki: conceptualization, formal analysis, writing – review and editing. Rémi Rabasa-Lhoret: conceptualization, formal analysis, writing – review and editing. Cathy J. Sun is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

ACKNOWLEDGEMENTS

We thank the Ottawa Methods Centre at The Ottawa Hospital Research Institute for their contribution to this publication. We appreciate the support from Risa Shorr, information specialist, for assistance with the literature search, and Lynn Gosselin-Mcrae, registered nurse, for performing the study protocol for the participants. We thank the study participants for their time and involvement.

FUNDING INFORMATION

This study was supported by The Ottawa Hospital Department of Medicine Pilot/Feasibility grant. CJS was supported by The Ottawa Hospital Department of Medicine Early Career Investigator Chair.

CONFLICT OF INTEREST STATEMENT

The authors have no potential conflicts relevant to this article.

14631326, 2025, 9, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16528 by National Health And Medical Research Council, Wiley Online Library on [29/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/dom.16528.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Cathy J. Sun https://orcid.org/0000-0002-4172-9784
Rémi Rabasa-Lhoret https://orcid.org/0000-0003-4706-5170

REFERENCES

1. Nolan CJ, Prentki M. Insulin resistance and insulin hypersecretion in the metabolic syndrome and type 2 diabetes: time for a conceptual framework shift. *Diab Vasc Dis Res*. 2019;16(2):118-127. doi:10.1177/1479164119827611
2. Bergman M, Abdul-Ghani M, DeFronzo RA, et al. Review of methods for detecting glycemic disorders. *Diabetes Res Clin Pract*. 2020;165:108233. doi:10.1016/j.diabres.2020.108233
3. Cersosimo E, Solis-Herrera C, Trautmann ME, Malloy J, Triplitt CL. Assessment of pancreatic β-cell function: review of methods and clinical applications. *Curr Diabetes Rev*. 2014;10(1):2-42. doi:10.2174/1573399810666140214093600
4. Kahn SE, Chen YC, Esser N, et al. The β cell in diabetes: integrating biomarkers with functional measures. *Endocr Rev*. 2021;42(5):528-583. doi:10.1210/endrev/bnab021
5. Kolic J, Sun WG, Cen HH, et al. Proteomic predictors of individualized nutrient-specific insulin secretion in health and disease. *Cell Metab*. 2024;36(7):1619-1633. doi:10.1016/j.cmet.2024.06.001
6. Mingrone G, Panunzi S, De Gaetano A, et al. Insulin sensitivity depends on the route of glucose administration. *Diabetologia*. 2020;63(7):1382-1395. doi:10.1007/s00125-020-05157-w
7. Watkins JD, Koumanov F, Gonzalez JT. Protein- and calcium-mediated GLP-1 secretion: a narrative review. *Adv Nutr*. 2021;12(6):2540-2552. doi:10.1093/advances/nmab078
8. Shankar SS, Vella A, Raymond RH, et al. Standardized mixed-meal tolerance and arginine stimulation tests provide reproducible and complementary measures of β-cell function: results from the foundation for the National Institutes of Health Biomarkers Consortium Investigative Series. *Diabetes Care*. 2016;39(9):1602-1613. doi:10.2337/dc15-0931
9. Van Cauter E, Mestrez F, Sturis J, Polonsky KS. Estimation of insulin secretion rates from C-peptide levels. Comparison of individual and standard kinetic parameters for C-peptide clearance. *Diabetes*. 1992;41(3):368-377. doi:10.2337/diab.41.3.368
10. Miquel-Kergoat S, Azais-Braesco V, Burton-Freeman B, Hetherington MM. Effects of chewing on appetite, food intake and gut hormones: a systematic review and meta-analysis. *Physiol Behav*. 2015;151:88-96. doi:10.1016/j.physbeh.2015.07.017
11. Cefalu WT, Franks PW, Rosenblum ND, et al. A global initiative to deliver precision health in diabetes. *Nat Med*. 2024;30(7):1819-1822. doi:10.1038/s41591-024-03032-4
12. Kössler T, Bobrov P, Strassburger K, et al. Impact of mixed meal tolerance test composition on measures of beta-cell function in type 2 diabetes. *Nutr Metab (Lond)*. 2021;18(1):47. doi:10.1186/s12986-021-00556-1

How to cite this article: Sun CJ, Lochnan H, Sorisky A, et al. A simplified protocol based on a standardized meal-stimulated beta-cell response to stratify people living with non-insulin-treated type 2 diabetes. *Diabetes Obes Metab*. 2025;27(9):5337-5341. doi:10.1111/dom.16528

14631326, 2025, 9, Downloaded from https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.16528 by National Health And Medical Research Council, Wiley Online Library on [29/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License