Preserved C-peptide in survivors of COVID-19: Post hoc analysis

Sophie A. Clarke MBBS1,2 | Maria Phylactou MBBS1,2 | Bijal Patel MBBS1 | Edouard G. Mills MBChB1 | Beatrice Muzi MSc1 | Chioma Izzi-Engbeaya MBBS1,2 | Bernard Khoo MBChB Cantab3 | Karim Meeran MBBS1,2 | Alexander N. Comninos MBBS1,2 | Ali Abbara MBBS1,2 | Tricia Tan MBChB1,4 | Nick Oliver MBBS1,2 | Waljit S. Dhillo MBBS1,2

1Division of Diabetes, Endocrinology and Metabolism, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK
2Department of Endocrinology, Imperial College Healthcare NHS Trust, London, UK
3Department of Endocrinology, Division of Medicine, Faculty of Medical Sciences, Royal Free Campus, University College London, London, UK
4Department of Clinical Biochemistry, Imperial College Healthcare NHS Trust, London, UK

Correspondence
Waljit S. Dhillo, MBBS, Section of Endocrinology and Investigative Medicine, Division of Diabetes, Endocrinology and Metabolism, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, W12 0NN, UK.
Email: w.dhillo@imperial.ac.uk

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1 | INTRODUCTION

Hyperglycaemia1 and new diagnoses of diabetes (including autoantibody-negative type 1 diabetes2) have been reported in patients with coronavirus disease 2019 (COVID-19).1 Moreover, 77% of patients with COVID-19 and ketoacidosis (a condition typically associated with type 1 diabetes or pancreatic destruction) had type 2 diabetes.3 The receptor necessary for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) cellular entry, ACE2, and its permissive protein, TMPRSS2, are present in the pancreatic microvasculature4 and, at postmortem, SARS-CoV-2 viral RNA has been detected in pancreatic beta cells.5 In islets infected with SARS-CoV-2, in vitro insulin secretion, particularly glucose-stimulated insulin secretion, is attenuated.6 Given this, it has been speculated that insulin secretory capacity may be curtailed by direct cytopathic action of SARS-CoV-2 on beta cells. However, it is unknown whether such changes are of clinical relevance in survivors of COVID-19.

Random non-fasting C-peptide (rCP) levels correlate with stimulated C-peptide levels derived during mixed meal tests,7 and have high sensitivity and specificity compared with the gold standard threshold of 600 pmol/L considered to indicate insulin deficiency.8 It remains to be determined whether there is evidence for deficient insulin secretion, consistent with beta cell destruction, in survivors of COVID-19. Therefore, we assessed rCP in patients at least 3 months after COVID-19 infection to assess longer term beta cell secretory capacity.

2 | METHODS

Ethical approval was provided by the London Bridge Research Ethics Committee (REC ref. 20/HRA/4110). This study was registered with the international standard randomised controlled trial number (ISRCTN) registry (ISRCTN15615697) and performed in accordance with the Declaration of Helsinki. All participants provided written informed consent prior to inclusion.

The study protocol has previously been described.9 Participants were survivors of COVID-19 aged 18 years or older diagnosed with COVID-19 and recruited from the hospital inpatient and outpatient population in the 3 months after discharge.
COVID-19 who attended Imperial College London NHS Healthcare Trust during March-November 2020, or who had responded to adverts placed on social media asking for patients who had tested positive for COVID-19 and then attended at least 3 months following diagnosis with COVID-19. Diagnosis of COVID-19 was confirmed using either real-time RT-PCR testing of a nasopharyngeal swab, confirmatory imaging (chest radiograph or CT scan), or a positive serum SARS-CoV-2 IgG antibody test after symptom onset. Exclusion criteria included those prescribed steroids following recovery from COVID-19, and those taking other medications known to affect cortisol-binding globulin (including the combined oral contraceptive pill, and hormone replacement therapy). Similarly, patients with underlying health conditions or states known to influence cortisol-binding globulin (including pregnancy, end-stage renal failure, or underlying malignancy) were also excluded.

Severity of COVID-19 was determined according to World Health Organization (WHO) classification.10 Serum glucose and insulin were measured using an Abbott Alinity ci-series analyser. The lower limits of detection were as follows: insulin 2.9 pmol/L, glucose 0.03 mmol/L. The inter-assay coefficients of variation were: insulin 2.1%, glucose 1%. The intra-assay coefficients of variation were: insulin 2.2%, glucose 1.2%. Serum C-peptide was measured using an Abbott Architect analyser. The lower limit of detection was 3.3 pmol/L, the inter-assay coefficient of variation was 4.0%, and the intra-assay coefficient of variation was 2.8%. Serum fructosamine was measured using the Roche Cobas kit on the Abbott Architect platform (colorimetric method); the intra-assay coefficient of variation was less than 0.5% at 299 and 256 μmol/L. The reference range was 205-500 μmol/L.

Serum antibodies to SARS-CoV-2 N protein (IgG) were measured using Abbott Architect assay. For those with an indeterminate result, additional testing for antibodies to SARS-CoV-2 spike protein (RBD) (IgG) was performed using Imperial Hybrid DABA.11

Study visits commenced 8:00 AM-9:30 AM and were non-fasted. Serum samples were taken for measurement of insulin, glucose, and C-peptide levels. Fifty-four of 55 patients (98%) had complete insulin, C-peptide, and glucose measurements (one participant had missing data for C-peptide levels and was excluded from further analysis).

Statistical analysis was performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA). Distribution of data was assessed by D’Agostino and Pearson normality tests. Data were presented as mean ± standard deviation (SD) if parametrically distributed, or median with interquartile range (IQR) if not. Student’s t-test, or one-way analysis of variation (ANOVA), was used to compare two or more groups respectively, of parametrically distributed data. Mann–Whitney U test, or Kruskal–Wallis test with post hoc Dunn’s test, was used to compare two or more groups respectively, of non-parametrically distributed data. Multivariable linear regression was used to assess the ability of selected variables to predict C-peptide, including age, sex, ethnicity, pre-existing diabetes, body mass index (BMI) and severity of COVID-19 disease, according to the WHO severity index.

3 | RESULTS

3.1 | Baseline characteristics

In total, 55 participants attended for assessment at a median of 233 days post-COVID-19 infection. Of these, 15 (27.8%) had pre-existing type 2 diabetes; none had type 1 diabetes. No participants received a new diagnosis of type 2 diabetes following COVID-19 infection (Table 1). All participants with pre-existing diabetes were on metformin but none were treated with insulin.

3.2 | C-peptide is preserved at follow-up at ≥3 months following presentation with COVID-19

At the study visit appointment, which took place at least 3 months after initial presentation with COVID-19, median (IQR) C-peptide was 1319 (849, 1905) pmol/L and no patients had undetectable C-peptide levels (<3.3 pmol/L). Of the study cohort, 14.8% (n = 8) had C-peptide levels of less than 600 pmol/L with normoglycaemic contemporaneous glucose concentrations of 4.7-5.7 mmol/L, none of whom had known diabetes (Figure 1A).

3.3 | C-peptide levels and pre-existing diabetes

C-peptide values at follow-up at least 3 months after initial presentation with COVID-19 compared with those without (median [IQR] C-peptide [pmol/L]: pre-existing diabetes 1550 [1285, 2638], no diabetes 1239 [675, 1622], P = .008) (Figure 1B). There was no relationship between C-peptide and pre-COVID-19 HbA1c levels for those for whom data were available (n = 12) (P = .81), or with convalescent fructosamine levels (P = .85) (Figure S1B).

3.4 | C-peptide and COVID-19 disease severity and management

C-peptide levels were higher in those who had severe or critical disease compared with those with mild disease (median [IQR] C-peptide mild: 762 [510, 1289] pmol/L, severe/critical: 1538 [1143, 2906] pmol/L, P = .004) (Figure 1C). These data remained similar when those with diabetes were excluded (median [IQR] C-peptide mild: 675 [503, 1152] pmol/L, severe/critical: 1510 [1007, 2906] pmol/L). There was no correlation between C-peptide and either peak CRP or procalcitonin levels during acute admission with COVID-19 infection for those for whom data were available (n = 25). Similarly, C-peptide levels were not related to admission glucose level for those for whom data were available (n = 26).

The majority (n = 33, 61.1%) did not receive steroid treatment for acute COVID-19. However, there was no relationship between
C-peptide levels and cumulative dexamethasone dose ($P = .32$) (Figure 1D). When ethnicity, sex, age, disease severity, BMI, and pre-existing diabetes were assessed using multivariable linear regression, only a history of diabetes predicted C-peptide at follow-up ($P = .001$). The proportion of participants with diabetes was not different between those with mild, moderate, or severe/critical disease ($P = .20$).

**4 | DISCUSSION**

Hyperglycaemia in patients with COVID-19 is associated with increased mortality rates, and results from both impaired insulin secretion and increased peripheral insulin resistance. However, the extent to which these causes of dysglycaemia persist beyond acute infection is unclear.

Both critical and viral illnesses increase peripheral insulin resistance, particularly in people with pre-existing metabolic disorders. Furthermore, the viruses themselves may contribute to beta cell destruction and dysfunction, with cytomegalovirus, Epstein–Barr virus, rotavirus, rubella virus, and enteroviruses (including coxsackievirus B) all being associated with pancreatic beta cell apoptosis and subsequent type 1 diabetes. Similarly, SARSCoV-2 induces pancreatic islet cell apoptosis and thus has the potential to result in beta cell destruction and resultant insulinopaenia. These data assess the clinical relevance of such findings.

In this analysis of 54 survivors of COVID-19, all participants (including those with pre-existing type 2 diabetes) had detectable C-peptide levels.
Although C-peptide levels were not associated with peak procalcitonin or CRP levels, C-peptide values were greater in participants with severe or critical disease compared with those with mild disease. Similar to data from national cohorts, BMI was also greater in those who had had critical disease compared with those with mild disease (Figure S1), which may explain this observation.

Although not all the participants received steroid treatment, C-peptide levels at follow-up were not different in those who had received dexamethasone. While the use of steroids probably contributes to insulin resistance during acute COVID-19, it does not appear to have a deleterious legacy effect on insulin secretion at follow-up.

Finally, while critical and viral illness-induced insulin resistance probably explains a large proportion of the dysglycaemia observed, given that C-peptide levels were preserved at follow-up, these data suggest that any insulinopaenia during acute COVID-19 is not apparent at 3 months or more after presentation. These data are consistent with findings from other studies where it has been shown that, while a minority of patients with acute COVID-19 display characteristics of beta cell failure, in contrast to other causes of acute respiratory distress syndrome, the predominant cause of hyperglycaemia observed in patients with acute COVID-19 results from insulin resistance. Furthermore, patients with COVID-19 have been observed to have reduced adiponectin levels, consistent with adipose tissue dysfunction, providing a potential mechanism for the insulin resistance observed.

Taken together, given the deleterious impact of hyperglycaemia on outcomes in patients with COVID-19, agents that improve insulin resistance, such as metformin or thiazolidinediones, could have clinical utility, and hold potential as a focus for future research.

This post hoc analysis of data prospectively collected from survivors of COVID-19 is limited in that participants were not fasted. However non-fasted C-peptide is reflective of stimulated C-peptide levels in other clinical settings, and can provide a measure of pancreatic islet cell insulin secretory capacity. While the study cohort is small, and subject to survivor bias, and therefore must be interpreted with caution, it offers initial insights into COVID-19-related hyperglycaemia, and some reassurance that while dysglycaemia may persevere post initial infection, islet cell insulin secretory capacity is not permanently impacted. Further research into the underlying pathophysiology of hyperglycaemia in patients with COVID-19 is underway;

![Figure 1](image-url)
however, we provide important data to aid clinicians managing survivors of COVID-19.

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CONFLICT OF INTEREST
The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS
S.A.C., T.T., N.O., and W.S.D. conceived and designed the study. S.A.C., M.P., B.P., E.G.M., B.M., and C.I.-E. undertook study visits and performed data collection. S.A.C., B.K., K.M., A.N.C., A.A., T.T., N.O., and W.S.D. undertook analysis of the data. S.A.C. prepared the manuscript and all the authors reviewed and adjusted the manuscript as necessary. W.S.D. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

PEER REVIEW
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DATA AVAILABILITY STATEMENT
The datasets generated during and/or analysed during the current study are not publicly available due to patient confidentiality but are available from the corresponding author on reasonable request.

ORCID
Chiona Izzi-Engbeaya https://orcid.org/0000-0001-7599-0166
Tricia Tan https://orcid.org/0000-0001-5873-3432
Waljit S. Dhillo https://orcid.org/0000-0001-5950-4316

REFERENCES
1. Heaney AI, Grif GD, Simon EL. Newly diagnosed diabetes and diabetic ketoacidosis precipitated by COVID-19 infection. Am J Emerg Med. 2020;249(1):e3-e4.
2. Hollstein T, Schulte DM, Schulz J, et al. Autoantibody-negative insulin-dependent diabetes mellitus after SARS-CoV-2 infection: a case report. Nat Metab. 2020;2(10):1021-1024.
3. Müller JA, Groß R, Conzelmann C, et al. SARS-CoV-2 infects and replicates in cells of the human endocrine and exocrine pancreas. Nat Metab. 2021;3(2):149-165.
4. Coate KC, Cha J, Shrestha S, et al. SARS-CoV-2 cell entry factors ACE2 and TMPRSS2 are expressed in the microvasculature and ducts of human pancreas but are not enriched in β cells. Cell Metab. 2020;32(6):1028-1040.
5. Fignani D, Licitra G, Brusco N, et al. SARS-CoV-2 receptor angiotensin I-converting enzyme type 2 (ACE2) is expressed in human pancreatic β-cells and in the human pancreas microvasculature. Front Endocrinol. 2020;11:596898.
6. Montefusco L, Ben Nasr M, D’Addio F, et al. Acute and long-term disruption of glycomeabolic control after SARS-CoV-2 infection. Nat Metab. 2021;3:774-785.
7. Wu C-T, Lidsky PV, Xiao Y, et al. SARS-CoV-2 infects human pancreatic β cells and elicits β cell impairment. Cell Metab. 2021;33:1565-1576.
8. Hope SV, Knight BA, Shields BM, Hattersley AT, McDonald TJ, Jones AG. Random non-fasting C-peptide: bringing robust assessment of endogenous insulin secretion to the clinic. Diabet Med. 2016;33(11):1554-1558.
9. Jones AG, Hattersley AT. The clinical utility of C-peptide measurement in the care of patients with diabetes. Diabet Med. 2013;30(7):803-817.
10. World Health Organization. Clinical management of COVID-19: interim guidance. World Health Organization; 2020.
11. Rosadas C, Randell P, Khan M, McClure MO, Tedder RS. Testing for responses to the wrong SARS-CoV-2 antigen? Lancet. 2020;396(10252):e23.
12. Clarke SA, Phylactou M, Patel B, et al. Normal adrenal and thyroid function in patients who survive COVID-19 infection. J Clin Endocrinol Metab. 2021;106:2208-2220.
13. Drucker DJ. Diabetes, obesity, metabolism and SARS-CoV-2 infection: the end of the beginning. Ann Oncol. 2020;33:479-498.
14. Bar-Or D, Rael LT, Madayag RM, et al. Stress hyperglycemia in critically ill patients: insight into possible molecular pathways. Front Med. 2019;6:1-6.
15. Op de Beeck A, Eizirik DL. Viral infections in type 1 diabetes mellitus - why the β cells? Nat Rev Endocrinol. 2016;12(5):263-273.
16. Pak C, Mcarthur R, Eun H-M, Yoon J-W. Association of cytomegalovirus infection with autoimmune type 1 diabetes. Front Med. 2019;6:1-14.
17. Fujiya A, Ochiai H, Mizukoshi T, et al. Fulminant type 1 diabetes mellitus associated with a reactivation of Epstein-Barr virus that developed in the course of chemotherapy of multiple myeloma. J Diabetes Investig. 2010;1(6):286-289.
18. Harrison LC, Perrett KP, Jachno K, Nolan TM, Honeyman MC. Does rotavirus turn on type 1 diabetes? PLoS Pathog. 2019;15(10):1-7.
19. Steenblock C, Richter S, Berger I, et al. Viral infiltration of pancreatic islets in patients with COVID-19. Nat Commun. 2021;12(1):3534.
20. Zhong F, Jiang Y. Endogenous pancreatic β cell regeneration: a potential strategy for the recovery of β cell deficiency in diabetes. Front Endocrinol. 2019;10:1-14.
21. Reiterer M, Rajan M, Gómez-Banoy N, et al. Hyperglycemia in acute COVID-19 is characterized by insulin resistance and adipose tissue infectivity by SARS-CoV-2. Cell Metab. 2021;33(11):2174-2188.
22. Zhu L, She ZG, Cheng X, et al. Association of blood glucose control and outcomes in patients with COVID-19 and pre-existing type 2 diabetes. Cell Metab. 2020;31(6):1068-1077.

SUPPORTING INFORMATION
Additional supporting information may be found in the online version of the article at the publisher’s website.

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