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Random non-fasting C-peptide: bringing robust assessment of endogenous insulin secretion to the clinic

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Abstract

Background—Measuring endogenous insulin secretion using C-peptide can assist diabetes management, but standard stimulation tests are impractical for clinical use. Random non-fasting C-peptide assessment would allow testing when a patient is seen in clinic.

Methods—We compared C-peptide at 90 minutes in the mixed meal tolerance test (sCP) with random non-fasting blood C-peptide (rCP) and random non-fasting urine C-peptide creatinine ratio (rUCPCR) in 41 participants with insulin-treated diabetes (median age 72 (IQR 68–78); diabetes duration 21 (14–31) years). We assessed sensitivity and specificity for previously reported optimal mixed meal test thresholds for severe insulin deficiency (sCP < 200 pmol/L) and Type 1 diabetes/inability to withdraw insulin (< 600 pmol/L), and assessed impact of concurrent glucose.

Results—rCP and sCP levels were similar (median 546 pmol/L and 487 pmol/L, $p=0.92$). rCP was highly correlated with sCP, $r=0.91$, $p<0.0001$, improving to $r=0.96$ when excluding samples with concurrent glucose < 8 mmol/L.

An rCP cut-off of 200 pmol/L gave 100% sensitivity and 93% specificity for detecting severe insulin deficiency, with area under the ROC curve 0.99. rCP < 600 pmol/L gave 87% sensitivity and 83% specificity to detect sCP < 600 pmol/L. Specificity improved to 100% when excluding samples with concurrent glucose < 8 mmol/L.

rUCPCR (0.52 nmol/mmol) was also well-correlated with sCP, $r=0.82$, $p<0.0001$. rUCPCR cut-off of < 0.2 nmol/mmol gave sensitivity and specificity of 83% and 93% to detect severe insulin deficiency, with ROC AUC 0.98.

Conclusions—Random non-fasting C-peptide measures are strongly correlated with mixed meal C-peptide, and have high sensitivity and specificity for identifying clinically relevant thresholds. These tests allow assessment of C-peptide at the point patients are seen for clinical care.

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Conflicts of interest:

None to declare

Introduction

Assessment of endogenous insulin secretion using C-peptide is useful in clinical practice to assist classification and treatment of diabetes (1). Assessment of a stimulated blood C-peptide level following a standardised stimulus such as a mixed meal (mixed meal tolerance test, MMTT) provides a gold-standard measure of endogenous insulin secretion, but is impractical for clinical use (2). Other C-peptide measures such as fasting blood C-peptide (3), or a post-home meal urinary C-peptide creatinine ratio (UCPCR)(4–6), give a reasonable approximation to the gold-standard, and high sensitivity and specificity in classifying diabetes (7–10). However, for routine clinical care, the most practical test would be a spot “random” non-fasting sample, sent when a patient is seen in an outpatient or primary care clinic.

Random non-fasting blood C-peptide (rCP) has been shown to have superior performance to both post-glucagon and fasting blood C-peptide assessment in differentiating clinically well-defined Type 1 and Type 2 diabetes (7, 8), and to have clinical utility in detecting MODY (11, 12). However rCP has never been formally validated against a gold-standard MMTT C-peptide assessment. While UCPCR changes little from 2 to 4 hours post-meal in those with Type 2 diabetes (McDonald, unpublished), utility of a random non-fasting UCPCR sample has never been assessed.

We aimed to compare non-fasting random blood C-peptide and UCPCR with ‘gold-standard’ blood C-peptide assessment at 90 minutes in the MMTT.

Methods

Participants

41 participants with insulin-treated diabetes were recruited to the GREAT study (<https://clinicaltrials.gov>, NCT02506296). To ensure a range of C-peptide values, participants were selected on the basis of prior C-peptide assessment to include participants with and without severe insulin deficiency (under/over 200pmol/L post-MMTT blood C-peptide or equivalent (1)). All participants had a clinical diagnosis of Type 2 diabetes, and an estimated glomerular filtration rate (eGFR) >30ml/min/1.73m². Ethical approval was obtained from the NRES Committee South West, and all participants gave written informed consent.

Mixed meal tolerance test

Participants fasted from 10pm, then attended the following day prior to 11am having not taken their morning medication prior to arrival. Baseline bloods for glucose and C-peptide were taken, morning insulin given (13), and 160ml of Fortisip Compact (Nutricia, Trowbridge, UK) drunk within 10 minutes (content/100ml: carbohydrate 29.7g, protein 9.6g, fat 9.3g). Bloods for C-peptide and glucose analysis were repeated every 30 minutes, up to and including 180 minutes post-mixed meal. Samples were immediately centrifuged after collection and stored at -80°C, for later batched analysis.

Non-fasting tests

On a separate occasion (within 8 days of the MMTT), blood was taken between 9am and 5pm, within 5 hours of a meal, and without restriction on snacks or other intake. Whole blood samples collected in potassium-EDTA (C-peptide) and fluoride oxalate (concurrent glucose) tubes were sent at room temperature to be processed routinely at the Royal Devon & Exeter Hospital Blood Sciences department. Participants were also asked to provide a spot urine sample. This was frozen at -80°C before later batch analysis.

Sample analysis

C-peptide was analysed using the automated Roche diagnostics (Manheim, Germany) E170 immuno-analyser (limit of detection 3.3pmol/L, inter- and intra-assay coefficients of variations <4.5% and <3.3% respectively). Urinary creatinine was analysed on the Roche P800 platform to obtain a urine C-peptide creatinine ratio (UCPCR, nmol/mmol).

Analysis

We compared the median random non-fasting blood C-peptide (rCP) with the median blood C-peptide at 90 minutes in the mixed meal tolerance test (sCP) using Wilcoxon's signed rank test, and correlation coefficient between both rCP and random non-fasting UCPCR (rUCPCR) with sCP using Spearman's rank correlation.

We then assessed the utility of rCP and rUCPCR in correctly classifying participants in relation to previously described clinically relevant MMTT C-peptide thresholds using receiver operating characteristic (ROC) curves, with corresponding specificities and sensitivities:

1. MMTT sCP <200pmol/L: absolute insulin deficiency (1, 14)
2. MMTT sCP <600pmol/L: Type 1 diabetes/inability to withdraw insulin (1)

Finally, we assessed the influence of concurrent glucose repeating the above analyses excluding hypoglycemia (concurrent glucose <4mmol/L), and a previously suggested cut-off of <8mmol/L (1, 8, 15).

Results

Participant characteristics

28/41 (68%) of participants were men. Participants had a median age of 73 (interquartile range, IQR 68-78), diabetes duration 21 (14-31) years, BMI 26.8 (25-29.9) kg/m², and HbA1c 68 (58-75) mmol/mol (8.4% (7.5-9.0%)).

12/41 (29%) participants had severe insulin deficiency (sCP<200pmol/L). C-peptide was detectable (>2.9pmol/L) at all time-points - fasting and stimulated - in 40/41 participants.

C-peptide was stable 1-3 hours after meal stimulation

There was little change in the C-peptide from 60 mins to 3 hours post-MMTT: median C-peptide ranged from 487 to 622pmol/L across these five time points, Figure 1a. Mean individual coefficient of variation over the 1-3 hour-post MMTT period was 14.3%.

Random non-fasting blood C-peptide level is strongly correlated with the gold-standard 90-minute mixed meal test C-peptide

Median rCP of 546 pmol/L (IQR 76-943) was similar to sCP at 90 minutes, 487 pmol/L (75-985), $p=0.92$, Figure 1a.

rCP was strongly correlated with sCP: Spearman's rho correlation coefficient=0.913, $p<0.0001$, Figure 1b. When only participants who had a concurrent lab glucose value of 8mmol/L were included (66% participants), the correlation coefficient increased to 0.96.

To be expected, results showed more variation in the higher C-peptide range, Supplementary Figure A.

Random non-fasting blood C-peptide is a highly sensitive and specific test for severe insulin deficiency

rCP was a highly sensitive and specific test for severe insulin deficiency ($sCP<200\text{pmol/L}$), with area under the ROC curve (AUC ROC) of 0.99 (95% confidence interval 0.91-1; Table 1). An rCP cut-off of $<200\text{pmol/L}$ gave a sensitivity of 100% (74-100) and specificity of 93% (77-99) for severe insulin deficiency, with 95% (83-99) of participants correctly classified. This did not alter significantly with concurrent glucose (Table 1).

rCP was also able to identify participants with $sCP<600\text{pmol/L}$ (Type 1 diabetes/inability to withdraw insulin): AUC ROC 0.94 (95% CI 0.84-0.99). An rCP value $<600\text{pmol/L}$ gave a sensitivity of 87% (66-97) and specificity of 83% (59-96) to detect $sCP<600\text{pmol/L}$ - with 85% (71-94) correctly classified. Excluding concurrent glucose values $<8\text{mmol/L}$ improved specificity to 100% (74-100) without altering sensitivity, Table 1.

Random non-fasting UCPCR is also strongly correlated with the gold-standard blood C-peptide measure and a sensitive and specific test for severe insulin deficiency

rUCPCR (median 0.52nmol/mmol (IQR 0.095-1.57nmol/mmol), was well-correlated with sCP, $r=0.82$, $p<0.0001$ ($n=40$). rUCPCR was also a sensitive and specific test for detecting the clinically relevant thresholds of $sCP<200\text{pmol/L}$ and $<600\text{pmol/L}$: ROC AUC 0.98 (0.87-1.0) and 0.90 (0.76-0.97) respectively, Table 1. For identifying severe insulin deficiency ($sCP<200\text{pmol/L}$), an rUCPCR cut-off of $<0.2\text{nmol/mmol}$ gave a sensitivity and specificity of 83% (52-98) and 93% (76-99), with 90% (76-97) participants being correctly classified. An rUCPCR cut-off of $<0.6\text{nmol/mmol}$ had a sensitivity and specificity of 82% (60-95) and 83% (56-96) for detecting $sCP<600\text{pmol/L}$.

Discussion

Our results show that random non-fasting blood C-peptide and UCPCR measurements taken when a patient attends clinic are highly correlated with the gold-standard mixed meal test assessment of endogenous insulin secretion, and are sensitive and specific tests for clinically relevant thresholds. These tests, combined with the demonstration of stability at room temperature of blood C-peptide for >24 hours (in EDTA (16)) and UCPCR for >72 hours (in boric acid (17)), offer a practical way of assessing endogenous insulin excretion when contact is made for clinical care.

Our findings are consistent with previous research demonstrating that a random non-fasting blood C-peptide offers similar performance to C-peptide in a formal glucagon stimulation test when classifying clinically well-defined Type 1 and 2 diabetes (8), is superior to fasting C-peptide when identifying autoimmune diabetes (7) and has high clinical utility for detecting patients with undiagnosed monogenic diabetes (11). This is the first study to formally evaluate use of a random non-fasting C-peptide sample against a gold-standard in a mixed meal test. The use of a random non-fasting UCPCR has not been previously assessed.

Limitations of our study include that our modest sample size limits our ability to assess the impact of concurrent glucose on random non-fasting C-peptide testing. In addition our population may not be representative of the patients where C-peptide testing has most utility (difficult to classify diabetes) in that they are older patients who have been selected on the basis of a clinical diagnosis of Type 2 diabetes with or without discordant C-peptide.

Our results suggest that a random non-fasting blood C-peptide or UCPCR could be used to assess endogenous insulin secretion in clinical practice. This would have major practical advantages in that the test can be conducted when a patient is seen for clinical care. While our sample size is too small to robustly assess the impact of concurrent glucose our results suggest this has only modest impact. While a high value in the presence of any glucose is likely to be robust it may be prudent to treat random non-fasting C-peptide values below a clinical threshold where concurrent glucose is <8mmol/L with caution, and consider a repeat sample.

Conclusion

Random non-fasting blood and urine C-peptide measures seem to be strongly correlated with the gold-standard C-peptide test and have high sensitivity and specificity in identifying clinically relevant C-peptide thresholds. A larger study would confirm findings, but these tests could allow assessment of C-peptide at the point patients are seen for clinical care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty Statement

- Measuring endogenous insulin secretion using C-peptide can assist diabetes management, but standard stimulation tests are impractical for clinical use
- This study assessed whether a random non-fasting C-peptide can be used to assess endogenous insulin secretion
- Random blood C-peptide and urine C-peptide creatinine ratio (UCPCR) were both highly correlated with mixed meal tolerance test C-peptide and were sensitive and specific measures for clinically useful mixed meal test thresholds
- A random non-fasting C-peptide taken when a patient is seen in clinic can be used to assess endogenous insulin secretion in clinical practice

Figure 1.

(a) Blood C-peptide levels on random sampling and in the mixed meal test. rCP: random non-fasting; time points reflect minutes post mixed meal ingestion, 0m: fasting sample. **(b)** Random non-fasting C-peptide versus 90 minute C-peptide in the mixed meal tolerance test. Level of blood glucose measured concurrently with rCP shown by blue diamonds >8 mmol/L; green circles >4 to 8 mmol/L; red triangles: <4 mmol/L.

Table 1

Ability of random non-fasting blood C-peptide (rCP) and UCPCR (rUCPCR) to define absolute insulin deficiency (90-minute mixed meal tolerance test C-peptide (sCP) <200pmol/L) and type 1 diabetes/insulin dependence (sCP <600pmol/L) using equivalent thresholds, with and without exclusion based on concurrent glucose (blood C-peptide only). Sensitivity, specificity and % correct classification are given for numerically equivalent thresholds (rCP 200 and 600pmol/L, UCPCR 0.2 and 0.6nmol/mol) as these were close to optimal on ROC analysis. 95% CI = 95% confidence intervals.

Mixed meal test C-peptide threshold	Concurrent glucose cut-off (mmol/L)	n	AUC	AUC 95% confidence intervals	Specificity 95% CI (%)	Sensitivity 95% CI (%)	Correctly classified 95% CI (%)
Random non-fasting blood C-peptide							
<200pmol/L	All	41	0.99	0.91–1.0	93 77-99	100 74-100	95 83-99
	4	39	1.0	0.91–1.0	96 82-100	100 72-100	97 87-100
	8	27	0.99	0.87–1.0	94 73-100	100 66-100	96 80-100
<600pmol/L	All	41	0.94	0.84–0.99	83 59-96	87 66-97	85 71-94
	4	39	0.94	0.79–0.98	83 59-96	86 64-97	85 69-94
	8	27	0.99	0.87–1.0	100 74-100	87 60-98	93 76-99
Random non-fasting UCPCR							
<200pmol/L	All	40	0.98	0.87–1.0	93 76-99	83 52-98	90 76-97
<600pmol/L	All	40	0.90	0.76–0.97	83 59-96	82 60-95	83 67-93