Transdermal blood sampling for C-peptide is a minimally invasive, reliable alternative to venous sampling in children and adults with type 1 diabetes

Running title: Transdermal C-peptide in type 1 diabetes

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Twitter summary: Blood sample collected through skin offers hope for virtually painless C-peptide testing in children and adults with #T1D #Type1diabetes type 1 #diabetes.

Abstract

Objective: C-peptide and islet autoantibodies are key type 1 diabetes biomarkers, typically requiring venous sampling, which limit their utility. We assessed transdermal capillary blood (TCB) collection as a practical alternative.

Research Design and methods: Ninety-one individuals (71 type 1 diabetes, 20 controls; type 1 diabetes: aged median 14.8 years[interquartile range 9.1-17.1]; diabetes duration 4.0 years[1.5-7.7]; controls 42.2 years[38.0-52.1]) underwent contemporaneous venous and TCB sampling for measurement of plasma C-peptide. Type 1 diabetes participants also provided venous serum and plasma, and TCB plasma for measurement of autoantibodies to glutamate decarboxylase, islet antigen-2, and zinc transporter 8. The ability of TCB plasma to detect significant endogenous insulin secretion (venous C-peptide ≥200pmol/L) was compared along with agreement in levels using Bland-Altman. Venous serum was compared with venous and TCB plasma for detection of autoantibodies using established thresholds. Acceptability was assessed by age-appropriate questionnaire.

Results: Transdermal sampling took a mean of 2.35minutes (SD 1.49). Median sample volume was 50 µl(IQR 40-50) with 3/91(3.3%) failures, and 13/88(14.7%) <35 µL). TCB C-peptide showed good agreement to venous plasma (mean venous ln(C-peptide) – TCB ln(C-peptide) = 0.008, 95% CI(-0.23, 0.29), with 100%(36/36) sensitivity/100%(50/50) specificity to detect venous C-peptide ≥ 200pmol/L. Where venous serum in multiple autoantibody positive TCB plasma agreed in 22/32

(sensitivity 69%), comparative specificity was 35/36 (97%). TCB was preferred to venous sampling (type 1 diabetes: 63% vs 7%; 30% undecided).

Conclusions: Transdermal capillary testing for C-peptide is a sensitive, specific, and acceptable alternative to venous sampling, TCB sampling for islet autoantibodies needs further assessment.

Article Highlights

- *Why did we undertake this study?*

We aimed to determine the precision and acceptability of transdermal capillary blood (TCB) for measurement of C-peptide.

- *What is the specific question(s) we wanted to answer?*

Is TCB a reliable and acceptable alternative to venous C-peptide measurement?

- *What did we find?*

From a study of 91 individuals (71 children and adults with type 1 diabetes, and 20 adult controls), TCB was found to be **a** sensitive, specific, and acceptable alternative to venous sampling.

- *What are the implications of our findings?*

TCB could be used as a reliable and practical alternative for C-peptide sampling.

C-peptide and pancreatic islet autoantibodies are key biomarkers used in type 1 diabetes. C-peptide reflects endogenous beta-cell function and is used in clinical care to aid in the correct classification of diabetes subtype (1-4). In research, C-

peptide is the primary outcome following interventions aiming to preserve beta-cell function (5). Islet autoantibody testing may be needed to confirm diabetes aetiology in clinical care and in research trials (4, 6), and can be used to identify children at risk of future clinically-diagnosed disease (7). The ability to accurately measure type 1 diabetes biomarkers, that is effective, painless, as well as acceptable, would be highly valuable, particularly in children.

C-peptide is typically collected using a venous blood draw, which is invasive involving a needle, which can be a challenge for young children, and restricts testing to the healthcare setting. Recently urine C-peptide: creatinine ratio (UCPCR) (8, 9), dried blood spot (DBS) C-peptide (10, 11) and more recently the volumetric absorptive microsampling device (VAMS)(12), have been shown to be practical alternatives to venous sampling. However, UCPCR requires an individual to void on demand, typically not possible in very young children. DBS C-peptide and the VAMS method are typically collected from a fingerstick blood spot using a lancet blade or needle, to produce a sample that is a mixture of arteriolar, venous and capillary blood. DBS C-peptide requires careful sample handling and processing, making it expensive, time consuming and difficult to measure C-peptide levels at very low concentrations, which are still clinically meaningful (13). Islet autoantibodies can be measured from serum taken from a venous or a capillary fingerstick or bloodspot sample, and which can be posted for analysis (14).

The Touch Activated Phlebotomy (TAP I) device has been developed for painless and minimally invasive blood collection (~100 µL), recently upgraded to collect a larger blood volume of ~300 µL, and has a Conformité Européene (CE) mark for measurement of glycated haemoglobin (HbA1c) in adults >21 years (15). The ‘CE’ mark status indicates that the device has been sold in the European Economic Area

and has been assessed to meet high standards and complies with European Union legislation. The TAP I/II and a different microsampling device (Tasso+) have now been tested successfully for use in a number of clinical and laboratory settings (16-23). However, the accuracy of using this method for measuring C-peptide or islet autoantibodies in type 1 diabetes has not been tested and there has been no assessment of acceptability of using this method in children. We therefore aimed to assess whether C-peptide and autoantibodies collected from transdermal capillary blood (TCB) from the TAP I device was a reliable and acceptable alternative to venous sampling.

RESEARCH DESIGN AND METHODS

Population

We studied 71 individuals with type 1 diabetes (defined by clinical diagnosis) and 20 adult controls (Table 1). Individuals with type 1 diabetes were recruited from paediatric and adult diabetes clinics at the John Radcliffe Hospital, and the Oxford Centre for Diabetes Endocrinology and Metabolism (OCDEM), Oxford, UK. Non-diabetic adult controls were recruited through poster advertisement at the John Radcliffe Hospital and OCDEM, as well as non-diabetic parents identified through patients attending the diabetes clinics involved in the study.

Recruitment was enriched for individuals with type 1 diabetes who had measurable C-peptide to allow assessment across a spread of C-peptide values, and we aimed to recruit at least 50% people with type 1 diabetes within 5 years of diagnosis. In order to assess acceptability, we aimed to recruit equal numbers of participants by age group, split between under 10 years 10-16 years and adults aged >16 years.

Participants were excluded if they had known renal impairment (eGFR<60ml/min/1.73 m^2), were pregnant, had a known coagulopathy, were on medication interfering with renal excretion and were non-English speakers. Parental consent along with assent was gained for children and young people aged under 16, and consent was gained for participants aged over 16 years.

Study Design

Initially we aimed to recruit 50 individuals with type 1 diabetes and 20 adult controls. Further individuals were recruited to replace the participants where sample collection was unsuccessful, defined as complete sample failure or collecting <35 µL plasma, which was anticipated to be the minimum viable volume of plasma needed for C-peptide measurement. Measurement of C-peptide was the primary aim of our study. Measurement of islet autoantibodies was a secondary aim to explore how much information a TCB sample could yield and therefore conducted after C-peptide analysis.

To minimise the impact of blood sampling for individuals with type 1 diabetes, sample collection was offered as part of their routine annual review visit, which would normally include a venous blood draw.

Topical anaesthetic was offered according to local policy for venous sampling. Prior to sampling, individuals with type 1 diabetes performed a self-monitoring blood glucose and sampling was delayed if blood glucose was <4 mmol/L, until resolution, to avoid C-peptide suppression.

Participants with type 1 diabetes had a 5 ml Li heparin plasma sample (for C-peptide measurement), and 1.3 ml serum-separating-tube sample (for autoantibody

measurement), and a concomitant TCB sample, collected by a researcher from the participants opposite upper arm. Control participants had only a 5 ml Li heparin plasma sample collected (for C-peptide measurement) with the concomitant TCB sample.

We recorded the time taken to collect the TCB sample, using the colour indicator turning from green to red on the TAP device. At the end of sample collection, the TAP device was removed, and the samples were centrifuged to allow plasma to be extracted and stored at -80^{0}C at the JDRF/Wellcome Diabetes and Inflammation Laboratory (DIL) at the Wellcome Centre for Human Genetics (WHG).

TAP I device

The TAP I Blood Collection® Device (manufacturer YourBio Health, Inc., USA, previously known as Seventh Sense Biosystems) combines capillary action with the use of 1mm long solid microneedles and vacuum extraction through the skin, to obtain 100 µL of capillary whole blood in LiHeparin anticoagulant (Supplemental Methods S1). A visual marker indicates when the device reservoir is filled (green to red). Following removal of the device from the skin, the blood sample is extracted via pipette method.

Laboratory methods

C-peptide

C-peptide samples were analyzed centrally. Plasma C-peptide was measured by electrochemiluminescence immunoassay (intraassay CV <3.3%; interassay CV <4.5%, assay limit 3.3 pmol/L) on a Roche Diagnostics (Mannheim, Germany) E170 analyzer by the Academic Department of Blood Sciences at the Royal Devon University National Health Service Healthcare Foundation Trust, Exeter, UK.

All samples underwent a minimum dilution with an equine proteinaceous diluent (Diluent Multi Analyte, Roche Diagnostics (Mannheim, Germany)) to achieve a minimum volume of 100 µL, required for analysis of C-peptide. Details of the dilutions performed can be found in Supplemental Table S1.

Islet autoantibodies

Plasma remaining after C-peptide measurement and undiluted venous serum samples, from participants with diabetes, were refrozen and sent to the Learning and Research Centre at Southmead Hospital, Bristol. Islet autoantibodies to GAD, IA-2 and ZnT8R/W were measured using established standardised radioimmunoassays with 125I or 35S labelled antigens (intra-assay CV 4%, 6% and 6%, inter-assay CV for a positive sample 20%, 19% and 21% measuring antibodies for GAD, IA-2 and ZnT8R/W, respectively) (24) (25). The sensitivity and specificity for these tests in Islet Autoantibody Standardisation Program 2020 was 64% and 97.8% for GADA, 72% and 100% for IA-2A, 60% and 100% for ZnT8RA and 56% and 100% for ZnT8WA, respectively.

Acceptability assessment

Usability of the TAP I device was assessed using a questionnaire, adapted from Lui et al (14). This included a traditional Likert scale, and a visual pain score (Wong-Baker Faces scale) (26). For participants aged under 16 years, the Likert scale was graded from 0 to 10, where 0 is ‘no hurt’ and 10 is ‘hurts worst’. For adult participants, the Likert scale was graded from 0 to 7, where 0 is ‘no pain’, 4 is ‘moderate pain’ and 7 is ‘very painful’, (Supplemental Methods S2). Participants aged over 16 years, competed the questionnaire independently, and for those aged under 16 years, it was completed by both participant and guardian. We further

recorded the choice of having a future test with either TAP I device or venous sample (TAP/Venous/don't mind).

Adverse events

Adverse events were recorded following venous and TAP device sampling over seven days. Data was recorded for redness, swelling and bruising (yes/no), and Pain was recorded on a scale of 0 to 4, where 0 was no pain, and 4 was spontaneously painful and prevents normal daily activities.

Statistical analyses

We assessed the time taken (minutes, seconds) to complete sample collection from the TAP device. We recorded the volume (in µL) of whole blood collected and the plasma extracted.

i. C-peptide

We compared the C-peptide of the venous sample with the TCB plasma sample using paired samples, where both measurements were available for each participant and excluded those where dilution resulted in raising the limit of detection so values could not be compared. Each venous sample had been divided into 4 or 5 aliquots, analysed separately for C-peptide, and the mean value was calculated for each participant, the standard deviation and range within each participant's measurements were calculated. The C-peptide value was log transformed. Using the paired values, the mean of the venous and TCB C-peptide, and the difference between the two were plotted. The Bland-Altman plot was used to assess the bias and the limits of agreement between the two methods.

We assessed the association between venous and TCB C-peptide (Pearson's correlation coefficient). We further assessed the ability of venous and TCB plasma C-peptide to correctly classify individuals with clinically-significant endogenous insulin production (defined as C-peptide ≥200pmol/L), with corresponding specificities and sensitivities.

ii. Islet autoantibodies

Thresholds for islet autoantibody positivity have been established using populations of people without diabetes as previously described and were 33 DK units/ml for GADA, 1.4 DK units/ml for IA-2A, and 1.8 units for ZnT8R/WA (24, 25).

Islet autoantibody levels were compared between venous serum with venous plasma, and separately with diluted TCB sample values.

Venous serum versus venous plasma

Venous serum samples (the gold standard) were compared to venous plasma samples using the Bland-Altman plot to assess the bias and the limits of agreement. Only observations where the level was great enough to be detected were included.

Venous serum versus TCB plasma

Results for venous serum and TCB plasma were log transformed and plotted to determine the impact of dilution on the precision of positive values. Sensitivity and specificity of plasma was compared with detection in serum, i.e. serum antibody positives were considered true positives, and serum antibody negatives true negatives. We assessed the ability of the TCB plasma to detect two or more islet autoantibodies, as is used in type 1 diabetes screening studies.

Acceptability

For participants aged under 16 years, pain scores were grouped as follows: 0 no pain, 2 mild pain, 4-6 moderate pain, 8-10 severe pain. For adults aged 16 years and over, scores were reported as follows: 1 no pain, 2-3 mild pain, 4-6 moderate pain, 7 severe pain.

Ethical considerations

This study was approved by the West Midlands - Edgbaston Research Ethics Committee, UK All subjects, and for those <16 years also their parents, gave informed consent.

RESULTS

Ninety-one individuals were recruited (71 with type 1 diabetes, 20 adult controls), Table 1. There were 20 participants aged under 10 years, 22 aged 10-16 years and 29 aged >16 years. Type 1 diabetes participants were aged median 14.8yr (IQR 9.1 – 17.7), range 1.2 – 41.0 years, with a diabetes duration of median 4.0 (1.5 – 7.7) years, range 0.1 – 23.0 years.

TCB sample collection

There were 3/91 (3.3%) absolute sample failures, and 13/88 (14.7%) with plasma volume (<35 µL). The absolute sample failures were all children (aged 11 months, 12 and 13 years), two were female and one was male. The samples yielding low plasma

volume were also all from children (median age 3.9 years, range 9 months to 9 years), five were female and eight were male. A median of 50 µl plasma (IQR 40-50 µl), range 10-65 µl was collected (n=88). Sample collection from 91 participants took a mean of 2.35 minutes (median 2.35), SD 1.49, range 0.37-7 minutes.

Relationship between TCB and venous samples

C-peptide

Bland Altman agreement

There were 372 venous samples in total, separated from multiple aliquots from the 91 participants: 81 samples from 20 healthy controls and 301 from 71 participants with type 1 diabetes. One hundred and thirty-nine samples had C-peptide <3 pmol/L, and all were from 34/71 participants with type 1 diabetes. The median value of the patient mean values was 45.5pmol/L (IQR <3, 626) and range <3 to 2792 pmol/L. There were 48 participants with both detectable C-peptide (>3 pmol/L) from paired TCB and venous plasma samples. Figure 1 shows the Bland Altman plot of these 48 paired examples, using the log transformed values. There is no statistically significant bias, the (mean venous ln(C-peptide) – TCB ln(C-peptide) = 0.008, 95% CI (-0.23, 0.29). The limits of agreement are -0.197, 0.213.

Transforming the values back to the original scale we can report the ratio of venous to TAP:

Mean=1.008 95% CI 0.79 to 1.34, limits of agreement 0.82 to 1.24.

Classification for C-peptide positivity

C-peptide in TCB plasma was highly correlated to venous serum (Figure 2), Pearson's correlation coefficient 0.996. There was 100% (36/36) sensitivity and 100% specificity (50/50) for significant endogenous beta cell function (>200 pmol/L) for the TCB compared with the venous sample. Table 2 reports the results of dichotomising C-peptide at 200 pmol/L for 86 participants.

Islet autoantibodies

Venous serum versus venous plasma

First, serum measurement (gold standard) was compared with venous plasma by Bland-Altman (Supplemental Figure S1A). Using serum measurement to determine true positive and negative, sensitivity was >89% and specificity >86% for all autoantibodies measured in venous plasma. Bland-Altman analysis identified mean biases of <1.3 (DK) units.

Venous serum versus TCB plasma (diluted)

Supplemental Figures S2A-D show the precision of TCB on islet autoantibody sampling. Sensitivity was 24/25(96%) for GADA, 32/39 (82% for IA-2A), 12/19 (63%) for ZnT8RA, and 10/20 (50%) for ZnT8WA. Specificity was 41/43 (95%), 27/29 (93%), 48/49 (98%) and 48/48 (100%), respectively.

Classification for Islet autoantibody positivity using TCB plasma

Islet autoantibody positivity is considered commonly in research studies and the presence of 2 or more islet autoantibodies is considered as a marker of early-stage type 1 diabetes, rather than the actual titre of the responses (27). The sensitivity and specificity for detecting two or more islet autoantibodies using TCB plasma was 68.8% (22/32) and 97.2% (35/36), respectively (Supplemental Table S3).

Acceptability

Usability

Likert scale.

Of the 71 participants 15 (21%) with type 1 diabetes reported no difference between venous and TAP sampling, 48/71 (68%) scored venous more painful than TAP, and 8/71 (11%) scored TAP more painful than venous (all by children and young people aged <16 years).

Type 1 diabetes: under 10 years

For children aged <10 years, 60% (n=12) reported no pain, 35% (n=7) mild/moderate pain and 5% (n=1) reported worst pain using the TAP device versus 50% (n=10), 40% (n=8) and 10% (n=2) after venous sampling, respectively (Supplemental Table S4A).

Type 1 diabetes: 10-16 years

For children aged 10-16years, 54% (n=12) reported no pain, 36% (n=8) mild/moderate pain and 9% (n=2) worst pain using the TAP device vs 27% (n=6), 59% (n=13) and 14% (n=3), after venous sampling, respectively, (Supplemental Table S4B).

Adults

Adults with type 1 diabetes, 79% (n=23) reported no pain, and 21% (n=6) mild pain using the TAP device vs 10% (n=3), 55% (n=16) mild, 31% (n=9) moderate and 3% (n=1) severe pain with venous sampling, Supplemental Table S4C. In adult controls (n=20), 100% reported mild pain vs 70% (n=14) mild pain and 30% (n=4) moderate pain on venous sampling, Supplemental Table S4D.

Patient preference

When asked to choose their preferred method for future sampling, the majority (63%, 44/70) stated they would prefer the TAP device versus 7% (5/70) venous sampling, and 30% (21/70) were undecided. This was similar across all age groups, with the highest preference to TAP seen in adults with type 1 diabetes (19/29, 65%; Supplemental Table S5).

Adverse event diaries

Redness on Day 1 was reported in 44% (32/73) for TAP device and 30% (21/69) for venous sampling (Supplemental Table S6). No analgesia was needed for either TAP or venous sample in the seven days following sampling.

CONCLUSIONS

We show that transdermal plasma C-peptide shows good agreement with venous plasma, and is a sensitive, specific and acceptable method to detect endogenous insulin secretion. Transdermal collection for islet antibodies needs further assessment.

Transdermal blood collection for C-peptide

Our results showed a strong agreement between paired venous and transdermal C-peptide. The precision of the device to identify significant endogenous insulin production (>200 pmol/L), makes it an attractive alternative to venous sampling. Although the transdermal method is not superior to venous sampling, it is more practical, since it does not involve a venous blood draw. Whilst transdermal sampling was more favourable in our study, it was not uniformly chosen by children. This can be explained by the standard method for collecting a venous blood draw in a children's hospital setting, which includes use of topical anaesthetic and access to play specialists. Allowing a reliable method to be undertaken without a venous blood draw may mean it could avoid bringing children into hospital unnecessarily, with an obvious cost saving. The main barrier to home testing would be the need to process the samples shortly after collection. This may be overcome by use of the next generation device (TAP II) that collects a greater blood volume directly into a blood collection tube containing an appropriate additive, that means it does not require immediate processing, a method recently tested in adults with rheumatic diseases with high acceptability (19). Since C-peptide is stable for at least 24 hours in plasma, home samples could be collected in EDTA plasma and brought to a community setting for transport to the local hospital laboratory for processing but would need further assessment.

Transdermal blood measurement for islet autoantibodies

The need to dilute the TCB samples in our study made the analysis of lower level autoantibody responses challenging, however the newer generation TAP II device would address this limitation.

We identified some differences between measurement in venous serum and TCB plasma samples. The lower specificity of IA-2A measured in TCB plasma is partially explained by a genuine difference between plasma and serum measurement in two participants; where IA-2A levels >15 DK units (more than ten times the threshold) in venous or TCB plasma compared with undetectable levels in serum, other differences were more subtle. This contrasts with previous work suggesting EDTA plasma and serum showed very high correlation for GADA and IA-2A measured by radioimmunoassay (28), but in that study fewer IA-2A were detected close to the threshold. The small difference between venous plasma and serum does not fully explain the lower sensitivity to detect individual islet autoantibodies using diluted TCB plasma. Even after a post-hoc adjustment for dilution (data not shown), levels did not agree. We hypothesise that the impact of transdermal sampling, with a high level of interstitial fluid and unknown matrix effect, may have affected the results. Overall sensitivity for detecting two or more islet autoantibodies was relatively low (69%), with high 97% specificity, suggesting further work is needed before using the transdermal methods to measure islet autoantibodies.

Alternative measures of capillary C-peptide

The strong relationship between transdermal blood collection for C-peptide with serum C-peptide is supported by previous studies assessing DBS compared to venous C-peptide during a mixed meal tolerance test (11). Both DBS and TCB C-peptide use capillary sampling. The method of extraction and processing is however different, with DBS requiring extraction of very small volumes that are not measurable at low levels. In contrast transdermal collection allows a larger volume to be processed and lower concentrations to be measured, although using this generation device with relatively low volumes requires dilution that will decrease the

limit of detection, and that has already been addressed using the TAP II device (19). Compared to a timed urine collection for measurement of UCPCR which requires an individual to void on demand (9), transdermal blood can be collected at any age and has been tested in infants as young as 2 months of age (18).

Strengths

The study included a large range of C-peptide and age ranges, making the results translatable to paediatric and adult type 1 diabetes settings.

Study limitations

Acceptability and pain were assessed, however no topical analgesic was used for TCB sample collection, so may be argued that it was not a fair comparison. Despite this, participants favoured use of the TAP device. In adults, who are not routinely offered topical analgesia for venous sampling, 63% (n=19) adults with type 1 diabetes favoured the TAP device, 7% (n= 2) the venous sample, and 27% (n=8) were undecided.

Insulin autoantibodies were not measured in this study owing to the relatively large volume of sample required, and after two weeks exogenous insulin injection can stimulate insulin antibody production. For use in research, particularly young children at risk of type 1 diabetes, including measurement of insulin autoantibodies would be preferable and tested on undiluted samples.

The absolute failure rate of sampling using the TAP device was only 3.3%, and 14.7% yielded a low plasma volume <35 µL). Cost was not assessed as part of this study, but device failure and the volume of sampling required would need to be accounted for in further routine analysis and before healthcare integration and home testing.

Implications

The TAP I device may have a role in home collection of C-peptide in the research setting in prospective studies assessing beta cell function and following interventions of disease modifying agents in both newly-diagnosed (Stage 3) as well as early-stage type 1 diabetes (Stages 1 and 2) (29). Since the gold standard measure of endogenous insulin secretion in type 1 diabetes, the mixed meal tolerance test, is costly and impractical, it is usually measured only 3-12 monthly following interventions in type 1 diabetes trials. Interim samples provide useful information, an approach adopted in our clinical trial in children with newly-diagnosed type 1 diabetes (30), with home collection of DBS C-peptide after a standardised meal. The transdermal approach has advantages over DBS C-peptide, mostly related to the method and volume of blood extracted, and may therefore offer the ability to measure C-peptide less invasively, more frequently and at lower assay limits. The current method is not sufficiently accurate for islet autoantibody testing.

Future work

The feasibility of collecting a home TAP I sample for measurement of C-peptide needs investigating. It may offer a potential practical alternative to the hospital testing. The feasibility and acceptability of collecting undiluted TAP samples for measurement of islet autoantibodies may have a role in screening for type 1 diabetes in the general population and first-degree relatives, in particular using the TAP II or Tasso+ device, and needs further assessment, since antibodies to rheumatic diseases have been successfully measured (19). The validation,

feasibility, and acceptability of home testing in other settings, in particular chronic diseases such as thyroid disease, would be worthwhile.

CONCLUSIONS

Transdermal blood collection may offer a precise and acceptable alternative to venous sampling for C-peptide in children and adults with type 1 diabetes. Further assessment is needed for home collection and for assessment of islet autoantibody measurement.

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Conflict of interest

R.E.J.B has received speaking honoraria from Eli Lilly and Springer Healthcare and sits on a voluntary basis as part of the NovoNordisk Research UK Foundation on the grant and fellowship selection committee. J.A.T. is a member of the Scientific Advisory Boards of GSK, Qlife, Precion and Vesalius Therapeutics. YourBio provided 50 free TAP devices for the study but contributed no intellectual design or analysis of the study.

Author Contributions and Guarantor Statement

R.E.J.B. conceptualised the study, researched data, wrote, review and edited the manuscript. A.L. researched data, contributed to discussion, reviewed, and edited the manuscript. T.J.M. researched data, contributed to discussion, reviewed, and edited the manuscript. K.R.O. contributed to recruitment and protocol development, reviewed and edited the manuscript. R.L. developed the protocol and recruited participants. C.W. and O.P. undertook sample analysis and reviewed and edited the manuscript. A.L. developed the protocol, researched data, contributed to discussion, reviewed, and edited the manuscript. C.S. reviewed and edited the manuscript. J.B. researched data, contributed to discussion, reviewed and edited the manuscript. J.A.T. developed the protocol, contributed to discussion, reviewed and edited the manuscript. All authors contributed to the discussion, reviewed the manuscript and approved the final version of the manuscript. R.E.J.B 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.

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	Type 1 diabetes,n=71	Controls,n=20
Age median(IQR)yr	14.8(9.1-17.7)	42.2(38.0-52.1)
Diabetes duration median (IQR)yr	4.0(1.5-7.7)	-
N(%) females	38/71(54%)	19/20(95%)
Ethnicity		
Asian/Asian British	2(3%)	0
Black/African/Caribbean/Black British	3(4%)	1(5%)
Mixed/Multiple Ethnic Groups	7(10%)	1(5%)
White	60(83%)	18(90%)

Table 1. Participant characteristics. Data presented as median (interquartile range), unless otherwise stated.

	Venous C-peptide ≥200pmol/L	Venous C-peptide <200pmol/L	Total
TCB C-peptide ≥200pmol/L	36	0	36
TCB C-peptide <200pmol/L	0	50	50
Total	36	50	86

Table 2. Categorisation of positive values, defined as C-peptide ≥200 pmol/L, in TCB plasma and venous serum samples.

Figure legends

Figure 1. Bland-Altman plot comparing the venous and TCB C-peptide measurements on 48 participants. The upper and lower horizontal lines show the limits of agreement.

Figure 2. Scatter diagram showing the relationship between random venous and TCB C-peptide in 20 controls and 71 type 1 diabetes participants (Pearson's correlation, r=0.996). Cut-off of C-peptide ≥200 pmol/L is shown with corresponding sensitivity and specificity.