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Title:

STIMULATED URINE C-PEPTIDE CREATININE RATIO VERSUS SERUM C-PEPTIDE FOR MONITORING OF BETA-CELL FUNCTION IN THE FIRST YEAR AFTER DIAGNOSIS OF TYPE 1 DIABETES

Running title: Urine versus serum C-peptide in the first year of Type 1 diabetes

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Novelty statement:

- Stimulated urine C-peptide/creatinine ratio can detect decline in beta-cell function in the first 12 months after diagnosis of type 1 diabetes

- In the first 6 months period after diagnosis there was poor correlation of the change in both stimulated serum and urine C-peptide with changes in metabolic measures (insulin dose adjusted HbA1c).

- It warrants inclusion in further prospective interventional and observations studies in type 1 diabetes.

ABSTRACT

Urine C-peptide/creatinine ratio measured after a mixed meal (MM-UCPCR) has been suggested as a less invasive alternative to the standard Mixed Meal Tolerance Test (MMTT) in assessing beta-cell function. There are limited data comparing these two measures soon after diagnosis of type 1 diabetes (T1DM), when beta-cell stress may affect insulin production.

Aims. To determine if UCPCR is a useful tool for monitoring beta-cell function in new-onset T1DM.

Methods. Data were obtained from a prospective immunomodulation study in people with T1DM ≤3 months from diagnosis with a standard MMTT and MM-UCPCR carried out at 0, 3, 6, 9 and 12 months. The change in the insulin dose adjusted HbA1c was correlated with the change in serum/urine C-peptide during the 12 months follow-up period.

Results. A significant reduction of MM-UCPCR was detected within 9 months. Stimulated serum and urine C-peptide responses did not correlate at baseline or 3 months in new onset participants, but did correlate after 6, 9 and 12 months. Neither the change in stimulated serum nor the urine C-peptide correlated with the change in insulin dose adjusted HbA1c in the first 6 months, but all measures correlated significantly at 9 and 12 months.

Conclusion. MM stimulated UCPCR is comparable to, although less sensitive than, stimulated serum C-peptide in monitoring beta-cell function during the first year from diagnosis. Since it is significantly less invasive, it warrants inclusion in further studies in T1DM and may represent an attractive alternative outcome measure in cohort studies and children.

INTRODUCTION

With increasing focus on immunomodulation(1-4) to preserve beta-cell function in T1DM, early identification of responders is of particular interest. People with impaired beta-cell function secrete smaller quantities of C-peptide in response to stimuli and exhibit a delay in reaching peak C-peptide(5). There is evidence of accelerated beta-cell damage around the time of diagnosis(6, 7). After resolution of glucotoxicity(8) and given that the beta-cell has limited potential for proliferation in recent-onset T1DM(9), it is possible that there may be diminished and erratic insulin/C-peptide production early after diagnosis, which may stabilise later.

Determining a reliable method to establish the efficacy of beta-cell restoration treatments is important. Most clinical trials use the Mixed Meal Tolerance Test (MMTT) as the gold standard to assess beta-cell function(10). A surrogate measure of beta-cell function, insulin dose adjusted HbA1c (IDAA1c) also correlates well with peak serum C-peptide 12 months after diagnosis(11). More recently, stimulated post-meal 2-hour urine C-peptide/creatinine ratio (UCPCR) has been proposed as an alternative and less invasive means of estimating beta-cell function(12). However, there are limited data comparing UCPCR and serum C-peptide measurements soon after diagnosis of T1DM and no prospective studies. Fasting UCPCR has been shown to be insensitive to capture changing insulin production in an immunointervention trial(13), however the finding that stimulated UCPCR can be used as a tool for monitoring beta-cell function in islet transplant patients suggests that it may be a useful outcome measure(14). Urine c-peptide samples are easy for

patients to send in samples by post, and combined with the less invasive nature of UCPCR testing makes it potentially very attractive as an outcome measure in large cohort/community studies and children.

Here we compare serial measurements of urine and serum C-peptide in people with newly diagnosed T1DM over a period of 12 months during an intervention trial.

METHODS

Setting and participants

This multi-centre, double-blinded randomised controlled intervention trial, was designed to assess the safety of C19-A3 proinsulin peptide administration and the change in stimulated C−peptide production between baseline and 12 months after treatment in adults with new-onset T1DM. The primary outcomes of this study are reported elsewhere (in submission). Twenty seven adults with T1DM of ≤3 months duration (time from the insulin treatment start to the initiation of study drug ≤100 days), were recruited from June 2013 to March 2014 from 5 UK centers. Participants were randomised into 3 groups: placebo (n=8, age 28.9±8.2 years, female:male=2:6), low-frequency treatment group (six 4-weekly peptide injections; n=10, age 26.6±5.5 years, female:male=4:6) and high-frequency treatment group (twelve 2-weekly peptide injections; n=9, age 30.0±5.7 years, female:male=3:6). The treatment period was 6 months followed by an observation period of 6 months. Participants received insulin injections as a part of standard clinical care.

The study was approved by South West 2 Research Ethics Committee (ClinicalTrials.gov Identifier NCT01536431, ISRCTN 66760879). All participants gave written informed consent.

Beta-cell stimulation methods

Ensure Plus® (Abbott Nutrition, 6ml/kg (max 360ml)) was used as a Mixed Meal (MM) stimulant of beta-cell production, in both the standard MMTT and stimulated urine C-peptide measurements.

The standard MMTT was carried out after overnight fast as described before(10) at 0, 3, 6, 9 and 12 months. Serum samples for C-peptide (sCP) and glucose were collected at -10, 0, 15, 30, 60, 90 and 120 min. Urine samples were collected from the second void in the morning (before MM) and 120 min after the MM (MM-UCPCR). All urine was collected between 0 and 120 minutes.

Urine and serum samples

Urine samples were collected into boric acid containers (Sterilin, Thermo Scientific) and transported to a laboratory at ambient temperature within 72h. If not assayed within 72 hours of collection they were stored at -80°C for up to 14 days until assay.

Serum samples were stored at -20°C and sent to the lab (in dry ice) in batches where they were assayed.

Assay methods

Urine C-peptide was measured, in samples diluted 1:10, by ELISA (Mercodia, UK). Detection limit for the C-peptide assay was 25 pmol/l with inter-assay CV of <5%. Urine creatinine was assayed by a colorimetric method (Jaffe reaction, Randox Ltd, UK). Detection limit and inter-assay CV% were 100 umol/l and ≤5.3% respectively. Results were expressed as UCPCR (nmol/mmol).

Serum C-peptide was measured by an immunochemiluminometric assay (Invitron, UK). Detection limit and inter-assay CV were 5pmol/l and ≤7.8% respectively.

Statistical analysis

Data are expressed as mean±SD. Non-normally distributed data are expressed as median with interquartile range. Differences were considered significant if *p* value was <0.05. GraphPad Prism version 4.0a for Macintosh was used for the analysis.

The area under the curve (AUC) was calculated using the trapezoidal method not adjusted for the baseline C-peptide but normalised for the 120 minutes period of the standard MMTT using the sCP value at each time point.

Insulin dose adjusted HbA1c (IDAA1c) was calculated according to the following formula: HbA1c (%) + [4 × insulin dose (units per kilogram per 24 h)].(11)

Non-parametric Spearman correlation was performed to correlate the AUC sCP during standard MMTT/stimulated UCPCR and IDAA1c (change comparing to baseline (Δ)). The strength of association between measures was assessed by correlation coefficient (r) and p value.

Wilcoxon signed ranked test was used to test the significance of percentage change in relation to the baseline value.

RESULTS

AUC sCP correlated with peak sCP during MMTT throughout the follow-up period (before: r=0.98; 3months: r=0.98; 6months: r=0.83, 9months: r=0.94, 12months: r=0.97; all p<0.0001) and is used as a comparison variable to the urine C-peptide for the measurement of beta-cell function.

A significant reduction of MM-UCPCR was reached at 9 months (-45.4%, p=0.03), whilst the reduction in AUC sCP reached significance after 3 months (-54.7%, p=0.008) in placebo treated participants, Table 1.

In the pooled analysis of placebo and treatment group, the change from baseline in AUC sCP did not correlate with the change in the corresponding MM-UCPCR after 3 months (r=0.17, p=0.48). However, a significant correlation was achieved after 6 months (r=0.56, p=0.007), 9 months (r=0.65, p=0.002) and 12 months (r=0.54, p= 0.02).

Consistently, neither the change in stimulated serum nor in urine C-peptide correlated with the change in IDAA1c in the first 6 months (MM-UCPCR - Figure 1a and b; MM-AUC sCP - Figure 1e and f). However, at 9 and 12 months, both variables reached significant correlation with IDAA1c (MM-UCPCR, 9 and 12 months: r=-0.60, p=0.02; r=-0.68, p=0.005, respectively, Figure 1c and d; MM-AUC sCP, 9 and 12 months: r=-0.64, p=0.002; r=-0.66, p=0.001, respectively, Figure 1g and h).

DISCUSSION

Although stimulated UCPCR correlates well with AUC sCP in people with established T1DM(12), there is no information on how UCPCR performs as a test around the time of diagnosis or in prospective assessment of beta-cell function decline. The non-invasive nature of UCPCR measurement(12), makes it suited to large epidemiology studies. Our data in adults with new-onset T1DM suggests that stimulated UCPCR can be used as a robust outcome marker to measure decline in beta-cell function over the first 12 (but not 6) months from diagnosis. It appears to have slightly less sensitivity to change than AUC sCP, potentially requiring a larger sample size, but this will need to be balanced against the advantages of convenience and acceptability.

Both UCPCR and AUC sCP correlated poorly with a clinical measure of beta-cell function, IDAA1c, and with each other in the first 6 months after diagnosis, but improved over the second half of the follow-up period. These findings are consistent with a report of serum analyses from the combined TrialNet studies in which correlation between peak serum C-peptide and IDAA1c strengthens as the two year follow-up of over sixty newly diagnosed participants progressed(15). The initial lack of correlation in the first 6 months in our cohort may either be specific to adults or due to limit power in the current study. However, Mortensen et al. observed that IDAA1c and serum C-peptide have comparable validity in defining partial remission of T1D not earlier than 3 months from diagnosis(11).

It is possible that the insulin production is affected by the increased beta-cell stress reported during the first weeks after diagnosis measured by markers such as proinsulin/C-peptide ratio(16) and beta-cell death(17). Furthermore, it was observed in participants in the placebo arm of the early ciclosporin studies, that that the proinsulin/C-peptide ratio does not normalise until 9 months after diagnosis(18). It is unlikely that higher beta-cell reserve observed at the beginning of the study had a significant influence, as another study in people post islet-cell transplant with higher C-peptide production showed clear correlation between rapidly improving beta-cell function and glycaemic control(19).

The study has several limitations. It is possible that the intervention in the treatment group may have had a beneficial influence on C-peptide production. However, to overcome this, the correlation between the change in the measurements within the same individual (IDAA1c and C-peptide), was assessed. Differences in gender (20) and baseline renal function (c-peptide excretion) should not impact on this measure. Changes in renal function during the study might have an effect but were not seen.

Our data provide promising evidence that serial measurements of stimulated UCPCR can detect the decline in beta-cell function after the first year from diagnosis, where this was not seen using fasting urine c-peptide in a larger study. Cross-sectional studies suggest that home post UCPCR samples correlate well with MMTT stimulated measures(12), and this may there represent an even more convenient measure for use in large scale community studies. Further prospective studies are required to confirm this.

CONFLICT OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest associated with this manuscript.

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TABLES

Table 1. Change in MM-UCPCR (n=7) and MM-AUC sCP (n=8) in placebo treated MonoPepT1De participants.

UCPCR (nmol/mmol)	0	3 months	6 months	9 months	12 months
Median	1.43	1.03	0.73	0.78	0.33
Min	0.60	0.40	0.28	0.16	0.007
Max	1.71	7.57	1.50	1.25	1.71
IQR*	1.06 to 1.54	0.99 to 3.60	0.35 to 1.07	0.28 to 0.92	0.11 to 1.25
p**		0.58	0.11	**0.03**	**0.047**
AUC C peptide ([nmol x min]/l)	**0**	**3 months**	**6 months**	**9 months**	**12 months**
Median	0.53	0.24	0.22	0.30	0.21
Min	0.30	0.08	0.10	0.08	0.05
Max	0.98	0.46	0.75	0.89	0.69
IQR*	0.37 to 0.80	0.19 to 0.35	0.13 to 0.44	0.12 to 0.35	0.15 to 0.47
p**		**0.008**	**0.02**	**0.01**	**0.02**

*IQR – interquartile range
** comparison to time 0

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FIGURE LEGENDS

Figure 1. Correlation of the change (Δ) in insulin dose adjusted HbA1c (IDAA1c) and stimulated serum and urine C-peptide responses collected during the follow up period. *a-d* ΔIDAA1c vs ΔMM-UCPCR: *a)* after 3 months (r=-0.24, 95% CI: -0.63 – 0.23, p=0.30, n=20); *b)* after 6 months (r=0.10, 95% CI:-0.38 – 0.54, p=0.68, n=19); *c)* after 9 months (r=-0.60, 95% CI: -0.85 – -0.13, p=0.02, n=16); *d)* after 12 months (r=-0.68, 95% CI:-0.88 – -0.26, p=0.005, n=16); *e-h* ΔIDAA1c vs ΔMM-AUC sCP collected during the follow up period: *e)* after 3 months (r=-0.03, 95% CI: -0.43 – 0.39, p=0.89, n=24); *f)* after 6 months (r=0.18, 95% CI:-0.24 – 0.54, p=0.38, n=25); *g)* after 9 months (r=-0.64, 95% CI: -0.85 – -0.27, p=0.002, n=20); *h)* after 12 months (r=-0.66, 95% CI:-0.85 – -0.31, p=0.001, n=20).

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