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Stimulated urine C-peptide creatinine ratio vs serum C-peptide level for monitoring of β -cell function in the first year after diagnosis of Type 1 diabetes

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What's new?

- Stimulated urine C-peptide/creatinine ratio can detect decline in β -cell function in the first 12 months after diagnosis of Type 1 diabetes.
- Stimulated urine sampling represents an alternative, less invasive means of monitoring residual insulin production in prospective studies such as trials and cohort studies.

Abstract

Aims To determine if urine C-peptide/creatinine ratio is a useful tool for monitoring β -cell function in new-onset Type 1 diabetes.

Methods Data were obtained from a prospective immunomodulation study in people with Type 1 diabetes ≤ 3 months from diagnosis, with a standard mixed-meal tolerance test and measurement of urine C-peptide/creatinine ratio carried out at 0, 3, 6, 9 and 12 months. The change in the insulin-dose-adjusted HbA_{1c} level was also correlated with the change in serum/urine C-peptide level during the 12-month follow-up period.

Results A significant reduction in urine C-peptide/creatinine ratio, measured after a mixed-meal, was reached at 9 months (-45.4%), whilst the reduction in stimulated serum C-peptide level reached significance after 3 months (-54.7%) in placebo-treated participants. Neither change in stimulated serum C-peptide nor change in urine C-peptide level correlated with each other, and nor did change in insulin-dose-adjusted HbA_{1c} level in the first 6 months, but all measures correlated significantly in the second half of the 12-month follow-up period.

Conclusion. Mixed-meal-stimulated urine C-peptide/creatinine ratio was similar to, although less sensitive than, stimulated serum C-peptide level in monitoring β -cell function during the first year after diagnosis. Because the former is significantly less invasive, it warrants inclusion in further studies in Type 1 diabetes and may represent an attractive alternative outcome measure in cohort studies and in children.

Introduction

With increasing focus on immunomodulation [1–4] to preserve β -cell function in Type 1 diabetes, early identification of responders is of particular interest. Impaired β -cell function results in delayed and blunted C-peptide responses to stimuli [5]. There is evidence of accelerated β -cell damage around the time of diagnosis [6,7]. After resolution of glucotoxicity [8] and given that β cells have limited potential for proliferation in recent-onset Type 1 diabetes [9], there may be diminished and erratic insulin/C-peptide production early after diagnosis, which may stabilize later.

Most clinical trials use a mixed-meal tolerance test (MMTT) to assess and monitor β -cell function [10]. A surrogate measure of β -cell function, insulin-dose-adjusted HbA_{1c} (IDAA_{1c}), correlates well with peak serum C-peptide level 12 months after diagnosis [11]. Stimulated post-meal 2-h urine C-peptide/creatinine ratio (urine C-peptide/creatinine ratio) has also been proposed as less invasive alternative means of estimating β -cell function [12]. There are limited data and no prospective studies comparing urine C-peptide/creatinine ratio and serum C-peptide measurements soon after diagnosis of Type 1 diabetes. Fasting urine C-peptide/creatinine ratio has been shown to be insensitive for the capture of changing insulin production in an immunointervention trial [13]; however, its use as a tool for monitoring β -cell function in islet transplant patients suggests that it may have utility [14]. The less invasive nature of urine C-peptide/creatinine ratio testing makes it potentially attractive as an outcome measure in large cohort/community studies.

We compared serial measurements of urine and serum C-peptide in adults with new-onset Type 1 diabetes over 12 months during an intervention trial.

Methods

This multicentre, double-blind randomized controlled intervention trial, was designed to assess the safety of C19-A3 proinsulin peptide and the change in stimulated C-peptide production between baseline and 12 months after treatment in adults with new-onset Type 1 diabetes. The primary outcomes of this study are reported elsewhere (in submission). A total of 27 adults with Type 1

diabetes of ≤ 3 months' duration (time from start of insulin to the initiation of study drug ≤ 100 days) were recruited between June 2013 and March 2014 from five UK centres. Participants were randomized into three groups: placebo ($n=8$, age 28.9 ± 8.2 years, women:men ratio =2:6), low-frequency treatment (six 4-weekly peptide injections; $n=10$, age 26.6 ± 5.5 years, women:men ratio =4:6) and high-frequency treatment (12 peptide injections every 2 weeks; $n=9$, age 30.0 ± 5.7 years, women:men ratio =3:6). The treatment period was 6 months, followed by a 6-month observation period. Participants received insulin injections as a part of standard clinical care.

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

Ensure Plus[®] [Abbott Nutrition, Maidenhead, UK; 6 ml/kg (max 360 ml)] was used as a mixed-meal stimulant of β -cell production, in both the standard MMTT and the assessment of stimulated urine C-peptide/creatinine ratio. The standard MMTT was carried out after overnight fast, as previously described [10], at 0, 3, 6, 9 and 12 months. Serum samples for C-peptide 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 MMTT) and 120 min after the MMTT (mixed-meal-urine C-peptide/creatinine ratio) with no urine loss in between.

Urine samples were collected in boric acid containers (Sterilin; Thermo Scientific, Newport, UK) and transported to a laboratory at ambient temperature within 72 h. If not assayed within 72 h of collection they were stored at -80°C for up to 14 days. Serum samples were stored at -20°C and transported on dry ice in batches.

Urine C-peptide level was measured in samples diluted 1:10, using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The detection limit for the C-peptide assay was 25 pmol/l, with intra- and inter-assay coefficients of variation of $<5\%$ and $<5\%$, respectively. Urine creatinine was assayed using a colorimetric method (Jaffe reaction; Randox Ltd, London, UK). The detection limit,

and intra- and inter-assay coefficients of variation were 100 umol/l, <4% and <6%, respectively. Results were expressed as urine C-peptide/creatinine ratio (nmol/mmol). Serum C-peptide was measured using an immunochemiluminometric assay (Invitron, Monmouth, UK). The detection limit, and intra and inter-assay coefficients of variation were 5pmol/l, <5% and <8%, respectively.

Statistical analysis

Data are expressed as mean \pm SD or 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 baseline C-peptide but normalized for the 120-min period of the standard MMTT using the serum C-peptide value at each time point.

The IDAA_{1c} was calculated according to the formula: HbA_{1c} (%) + [4 \times insulin dose (units per kg per 24 h)] [11].

Non-parametric Spearman correlation was performed to correlate the AUC serum C peptide during standard MMTT/stimulated urine C-peptide/creatinine ratio and IDAA_{1c} [change comparing with baseline (Δ)]. The strength of association between measures was assessed using correlation coefficients (*r*), slope and *P* values. The absolute decline in urine C-peptide/creatinine ratio and AUC for C-peptide was analysed in the placebo group only to exclude treatment effects; correlations with IDAA_{1c} were made using change from baseline in all participants.

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

Results

The AUC for serum C-peptide correlated with peak serum C-peptide during the MMTT throughout the follow-up period (before: $r=0.98$; 3 months: $r=0.98$; 6 months: $r=0.83$; 9 months: $r=0.94$, 12 months: $r=0.97$; all $P<0.0001$) and was used as a comparison variable for urine C-peptide.

A significant reduction in mixed-meal-stimulated urine C-peptide/creatinine ratio was reached at 9 months (-45.4% , $P=0.03$), whilst the reduction in AUC for serum C-peptide reached significance after 3 months (-54.7% , $P=0.008$) in placebo-treated participants, Table 1.

In the pooled analysis of the placebo and treatment groups, the change from baseline in AUC for serum C-peptide did not correlate with the change in the corresponding urine C-peptide/creatinine ratio after 3 months ($r=0.17$, $P=0.48$). 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 ($r=0.54$, $P=0.02$).

Neither change in stimulated serum C-peptide nor in urine C-peptide level correlated with change in IDAA_{1c} in the first 6 months [mixed-meal-stimulated urine C-peptide/creatinine ratio (Fig. 1a,b); mixed-meal AUC for serum C-peptide (Fig. 1e,f)]. At 9 and 12 months, both variables correlated significantly with IDAA_{1c} [mixed-meal-stimulated urine C-peptide/creatinine ratio, 9 and 12 months: $r=-0.60$, $P=0.02$; $r=-0.68$, $P=0.005$, respectively (Fig. 1c,d); mixed-meal AUC for serum C-peptide, 9 and 12 months: $r=-0.64$, $P=0.002$; $r=-0.66$, $P=0.001$, respectively (Fig. 1g,h)].

Discussion

Although stimulated urine C-peptide/creatinine ratio correlates with AUC for serum C-peptide in people with established Type 1 diabetes [12], there is no information on how urine C-peptide/creatinine ratio performs as a test around the time of diagnosis or in prospective assessment of β -cell function decline. Our data on the placebo-treated participants with new-onset Type 1 diabetes only (independent of intervention) suggests that stimulated urine C-peptide/creatinine ratio can be a

valuable outcome marker to measure decline in β -cell function over the first 12 (but not 6) months from diagnosis (Table 1). Urine C-peptide/creatinine ratio appears to have slightly less sensitivity to change than AUC for serum C-peptide, potentially requiring a larger sample size, but this needs to be balanced against the advantages of convenience and acceptability.

Both urine C-peptide/creatinine ratio and AUC for serum C-peptide correlated poorly with a clinical measure of β -cell function, IDAA_{1c}, and with each other in the first 6 months, 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 level and IDAA_{1c} strengthens as the 2-year follow-up of >60 newly diagnosed participants progressed [15]. Buckingham *et al.* [15] observed a correlation with IDAA_{1c} in the first 6 months after diagnosis in children, suggesting that the initial lack of correlation in the first 6 months in the present cohort may either be specific to adults or attributable to limited power in the present study combined with an attenuating effect of the intervention. However, Mortensen *et al.* [11] observed that IDAA_{1c} and serum C-peptide had similar validity in defining partial remission of Type 1 diabetes not earlier than 3 months from diagnosis. Poor glycaemic control before the diagnosis can certainly influence baseline HbA_{1c} and IDAA_{1c}. With participants entering the study within 100 days from diagnosis, this is less likely to be the case in this study, with mean starting HbA_{1c} of 57.35 ± 12.60 mmol/l.

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

The present study has several limitations. It is possible that the intervention in the treatment group may have had a beneficial influence on C-peptide production. To overcome this, however, the correlation between the change in the measurements (IDAA_{1c} and C-peptide) within the same individual was assessed. Differences in gender [20] and baseline renal function (C-peptide excretion) should not have an 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 urine C-peptide/creatinine ratio can detect the decline in β -cell function during the first year after diagnosis, while this was not seen using fasting urine C-peptide in a larger study [13]. Cross-sectional studies suggest that home post-meal urine C-peptide/creatinine ratio samples correlate well with MMTT-stimulated measures [12], and may thus be a convenient measure in large-scale community studies.

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Competing interests

None declared.

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References

1. Herold KC, Gitelman SE, Ehlers MR, Gottlieb PA, Greenbaum CJ, Hagopian W *et al.* Teplizumab (Anti-CD3 mAb) Treatment Preserves C-Peptide Responses in Patients With New-Onset Type 1 Diabetes in a Randomized Controlled Trial: Metabolic and Immunologic Features at Baseline Identify a Subgroup of Responders. *Diabetes* 2013; **62**: 3766--3774.
2. Orban T, Bundy B, Becker DJ, DiMeglio LA, Gitelman SE, Goland R *et al.* Co-stimulation modulation with abatacept in patients with recent-onset type 1 diabetes: a randomised, double-blind, placebo-controlled trial. *Lancet* 2011; **378**: 412--419.

3. Rigby MR, Harris KM, Pinckney A, DiMeglio LA, Rendell MS, Felner EI *et al.* Alefacept provides sustained clinical and immunological effects in new-onset type 1 diabetes patients. *J Clin Invest* 2015; **125**: 3285--3296.
4. Haller MJ, Gitelman SE, Gottlieb PA, Michels AW, Rosenthal SM, Shuster JJ *et al.* Anti-thymocyte globulin/G-CSF treatment preserves beta cell function in patients with established type 1 diabetes. *J Clin Invest* 2015; **125**: 448--555.
5. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H *et al.* C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve beta-cell function: report of an ADA workshop, 21-22 October 2001. *Diabetes* 2004; **53**: 250--264.
6. Greenbaum CJ, Beam CA, Boulware D, Gitelman SE, Gottlieb PA, Herold KC *et al.* Fall in C-peptide during first 2 years from diagnosis: evidence of at least two distinct phases from composite Type 1 Diabetes TrialNet data. *Diabetes* 2012; **61**: 2066--2073.
7. Sosenko JM, Skyler JS, Beam CA, Krischer JP, Greenbaum CJ, Mahon J *et al.* Acceleration of the loss of the first-phase insulin response during the progression to type 1 diabetes in diabetes prevention trial-type 1 participants. *Diabetes* 2013; **62**: 4179--4183.
8. Chmelova H, Cohrs CM, Chouinard JA, Petzold C, Kuhn M, Chen C *et al.* Distinct roles of beta-cell mass and function during type 1 diabetes onset and remission. *Diabetes* 2015; **64**: 2148--2160.
9. Willcox A, Richardson SJ, Bone AJ, Foulis AK, Morgan NG. Evidence of increased islet cell proliferation in patients with recent-onset type 1 diabetes. *Diabetologia* 2010; **53**: 2020--2028.
10. Greenbaum CJ, Mandrup-Poulsen T, McGee PF, Battelino T, Haastert B, Ludvigsson J *et al.* Mixed-meal tolerance test versus glucagon stimulation test for the assessment of beta-cell function in therapeutic trials in type 1 diabetes. *Diabetes Care* 2008; **31**: 1966--1971.

11. Mortensen HB, Hougaard P, Swift P, Hansen L, Holl RW, Hoey H *et al.* New definition for the partial remission period in children and adolescents with type 1 diabetes. *Diabetes Care* 2009; **32**: 1384--1390.
12. Besser RE, Ludvigsson J, Jones AG, McDonald TJ, Shields BM, Knight BA *et al.* Urine C-peptide creatinine ratio is a noninvasive alternative to the mixed-meal tolerance test in children and adults with type 1 diabetes. *Diabetes Care* 2011; **34**: 607--609.
13. Ambery P, Donaldson J, Parkin J, Austin J. Urinary C-peptide analysis in an intervention study: experience from the DEFEND-2 oteelixizumab trial. *Diabet Med* Feb 12. doi: 10.1111/dme.13095. [Epub ahead of print]
14. Oram RA, Brooks AM, Forbes S, Eckoldt S, Smith RM, Choudhary P *et al.* Home urine C-peptide creatinine ratio can be used to monitor islet transplant function. *Diabetes Care* 2014; **37**: 1737--1740.
15. Buckingham B, Cheng P, Beck RW, Kollman C, Ruedy KJ, Weinzimer SA *et al.* CGM-measured glucose values have a strong correlation with C-peptide, HbA1c and IDAAC, but do poorly in predicting C-peptide levels in the two years following onset of diabetes. *Diabetologia* 2015; **58**:1167--1174.
16. Watkins RA, Evans-Molina C, Terrell JK, Day KH, Guindon L, Restrepo IA *et al.* Proinsulin and heat shock protein 90 as biomarkers of beta-cell stress in the early period after onset of type 1 diabetes. *Transl Res* 2015; **168**: 96--106.
17. Lehmann-Werman R, Neiman D, Zemmour H, Moss J, Magenheimer J, Vaknin-Dembinsky A *et al.* Identification of tissue-specific cell death using methylation patterns of circulating DNA. *Proc Natl Acad Sci USA* 2016; **113**:1826--1834.
18. Snorgaard O, Hartling SG, Binder C. Proinsulin and C-peptide at onset and during 12 months cyclosporin treatment of type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1990; **33**: 36--42.

19. Brooks AM, Oram R, Home P, Steen N, Shaw JA. Demonstration of an intrinsic relationship between endogenous C-peptide concentration and determinants of glycemic control in type 1 diabetes following islet transplantation. *Diabetes Care* 2015; **38**:105--112.
20. Thomas NJ, Shields BM, Besser RE, Jones AG, Rawlingson A, Goodchild E *et al.* The impact of gender on urine C-peptide creatinine ratio interpretation. *Ann Clin Biochem* 2012; **49**(Pt 4): 363--368.

Figure 1. Correlation of the change (Δ) in insulin-dose-adjusted HbA_{1c} (IDAA_{1c}) and stimulated serum C-peptide and urine C-peptide responses collected during the follow-up period. Δ IDAA_{1c} vs Δ mixed-meal-urine C-peptide/creatinine ratio (UCPCR) (a) after 3 months ($r=-0.24$, 95% CI -0.63 to 0.23, slope: -1.89 ± 3.46 ; $P=0.30$, $n=20$), (b) after 6 months ($r=0.10$, 95% CI -0.38 to 0.54, slope: 0.74 ± 0.76 , $P=0.68$, $n=19$), (c) after 9 months ($r=-0.60$, 95% CI -0.85 to -0.13, slope: -2.92 ± 1.52 , $P=0.02$, $n=16$) and (d) after 12 months ($r=-0.68$, 95% CI: -0.88 to -0.26, slope: -1.66 ± 0.60 , $P=0.005$, $n=16$). Δ IDAA_{1c} vs Δ mixed meal-area under the curve for serum C-peptide collected during the follow-up period (e) after 3 months ($r=-0.03$, 95% CI -0.43 to 0.39, slope: -0.55 ± 0.63 , $P=0.89$, $n=24$), (f) after 6 months ($r=0.18$, 95% CI -0.24 to 0.54, slope: 0.37 ± 0.42 , $P=0.38$, $n=25$), (g) after 9 months ($r=-0.64$, 95% CI -0.85 to -0.27, slope: -1.92 ± 0.54 , $P=0.002$, $n=20$) and (h) after 12 months ($r=-0.66$, 95% CI -0.85 to -0.31, slope: -1.09 ± 0.29 , $P=0.001$, $n=20$).

Table 1 Change in mixed meal-urine C-peptide/creatinine ratio ($n=7$) and mixed meal-area under the curve for serum C-peptide ($n=8$) in placebo-treated participants in the MonoPepT1De study.

	Month				
Urine C-peptide/creatinine ratio, nmol/mmol	0	3	6	9	12
Median	1.43	1.03	0.73	0.78	0.33
Minimum	0.60	0.40	0.28	0.16	0.007
Maximum	1.71	7.57	1.50	1.25	1.71
IQR	1.06–1.54	0.99–3.60	0.35–1.07	0.28–0.92	0.11–1.25
P^*		0.58	0.11	0.03	0.047
AUC C peptide ([nmol x min]/l)	0	3	6	9	12
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	0.37–0.80	0.19–0.35	0.13–0.44	0.12–0.35	0.15–0.47
P^*		0.008	0.02	0.01	0.02

AUC, area under the curve; IQR, interquartile range.

*Comparison with time 0.

Figure 1

