

## SUPPLEMENTARY DATA

### Supplementary Methods

#### Patients

We studied 72 patients with Type 1 diabetes (defined as age of diagnosis <30 years and on insulin since diagnosis). Patient characteristics are described in Tables S1 and S2. Patients were made up of 51 patients ≥18 years identified from routine diabetes clinics in Exeter, UK, and 21 children and young people with Type 1 diabetes <19 years from the pediatric clinic, University Hospital, Linköping, Sweden.

To enrich for patients who had endogenous insulin secretion 31/72 (43%) patients were either within 5 years of diagnosis or known to still secrete C-peptide when previously tested.

Patients were excluded from the study if they had known renal impairment (eGFR<60ml/min/1.73m<sup>2</sup>), severe hypoglycaemia within the last 3 months defined as an episode requiring third party assistance, documented hypoglycaemia unawareness with a blood glucose <3mmol/L, and HbA1c >10%.

#### Mixed Meal Tolerance Test (MMTT)

The MMTT commenced before 10:00 hrs following an overnight fast from at least midnight. Subjects on subcutaneous insulin injections withheld their morning short and long acting insulin, those on a subcutaneous insulin pump continued their background basal rate, according to previous protocols (1). The MMTT was rescheduled if the capillary glucose was <4mmol/l (<72mg/dl) or >15mmol/l (>270mg/dl).

#### Urine sample collection

##### MMTT

Patients had passed urine on waking on the test day and discarded the sample. Urine was collected immediately before the start of the test (as the second fasting morning void) and after 120 minutes. Any additional samples patients voided between 0 and 120 minutes were collected in full and added to the 120 minute void before aliquoting the 120 minute sample for analysis.

##### Home urine samples

Urine was collected in boric acid 120minutes after the evening meal following a void immediately pre-meal. Samples were brought to the research centre within 24 hours and aliquoted and frozen at -80°C.

Adult patients collected two further home 120minute postprandial urine samples, one after a standard 60g carbohydrate breakfast (2 toast, margarine, jam, 250ml fruit juice carton), and another following the patients' own lunch. Samples were brought to the research centre within 24 hours and aliquoted and frozen at -80°C.

#### Ethical Considerations

This study was approved by the South West Research Ethics Committee, UK and the Regional Research Ethics Committee, Linköping University, Sweden. All subjects, and for those <18 years also their parents, gave informed consent.

#### Laboratory methods

All C-peptide samples were analysed centrally. Urinary and serum C-peptide were measured by electrochemiluminescence immunoassay (intraassay CV <3.3%; interassay CV <4.5%) on a Roche Diagnostics (Mannheim, Germany) E170 analyzer by the Biochemistry department at the Royal Devon and Exeter National Health Service Foundation Trust, Exeter, UK. Urine creatinine was analyzed on the

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Roche P800 platform using creatinine Jaffé reagent (standardized against ID-MS) to obtain a urine C-peptide creatinine ratio (nmol/mmol).

### Supplementary Results

The UCPCR values, their correlations with the 90 minute serum C-peptide in the MMTT and the UCPCR cut off values that were derived from linear regression equations are given in Table S3 for the combined adult and paediatric cohort, and separately in Tables S4 and S5.

The standard breakfast and lunch performed similarly in adult subjects (see On-line appendix Table S6).

**Supplementary Table 1. Patient characteristics of adult cohort (n=51).** Data presented as median (interquartile range), unless otherwise stated.

|  | <b>Patient characteristics (n=51)</b> |
|--|---------------------------------------|
| <b>n (% male)</b>                      | 26(51)                                |
| <b>Age of diagnosis/y</b>              | 18(13-24)                             |
| <b>Current age/y<br/>(range)</b>       | 41.9(26.0-53.3)<br>18.2-81.9          |
| <b>Diabetes duration/y<br/>(range)</b> | 21.4(2.8-41.0)<br>(0.2-65.9)          |
| <b>BMI (kg/m<sup>2</sup>)</b>          | 24.7(22.0-27.2)                       |
| <b>HbA1c (%)</b>                       | 7.8(6.9-9.0)                          |

**Supplementary Table 2. Patient characteristics of pediatric cohort (n=21).** Data presented as median (interquartile range), unless otherwise stated.

|  | <b>Patient characteristics (n=51)</b> |
|--|---------------------------------------|
| <b>n (% male)</b>                      | 7(33.3)                               |
| <b>Age of diagnosis/y</b>              | 10.6(9.5-12.6)                        |
| <b>Current age/y<br/>(range)</b>       | 13.9(10.9-16.4)<br>(4.9-19.1)         |
| <b>Diabetes duration/y<br/>(range)</b> | 2.6(0.6-5.0)<br>(0.2-6.5)             |
| <b>BMI SDS score</b>                   | 0.31(-0.49-1.08)                      |
| <b>HbA1c (%)</b>                       | 7.2(6.6-7.9)                          |

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**Supplementary Table 3. UCPCR measurements and correlations with 90minute serum C-peptide during a MMTT in 72 children and adults with Type 1 diabetes.**

|   | UCPCR (nmol/mmol) |                  |                   |
|---|-------------------|------------------|-------------------|
|   | 0 minute          | 120 minute       | Evening meal      |
| <b>UCPCR median (IQR) nmol/mmol</b>   | 0.08 (0.01-0.35)  | 0.35 (0.04-1.41) | 0.16* (0.01-0.76) |
| <b>Spearman’s rank correlation, r (p value) with 90 minute serum C peptide in MMTT</b>          | 0.94 (<0.0001)    | 0.97 (<0.0001)   | 0.91 (<0.0001)    |
| <b>Regression equation UCPCR cut offs for serum C-peptide <math>\geq 0.2</math>nmol/mmol</b>    | 0.15              | 0.53             | 0.37              |
| <b>Sensitivity/specificity for detecting serum C-peptide <math>\geq 0.2</math>nmol/mmol (%)</b> | 84/97             | 94/100           | 84/97             |
| <b>Sensitivity/specificity (%) for UCPCR 0.53nmol/mmol</b>                                      |                   |                  | 71/97             |

\*120minute MMTT UCPCR v home evening meal, p<0.0001

**Supplementary Table 4. UCPCR measurements and correlations with 90minute serum C-peptide during a MMTT, in adults with Type 1 diabetes (n=51).**

|  | UCPCR (nmol/mmol) |                  |                 |
|--|-------------------|------------------|-----------------|
|  | 0 minute          | 120 minute       | Evening meal    |
| <b>UCPCR median (IQR) nmol/mmol</b>  | 0.05(0.01-0.41)   | 0.09(0.03-1.49)* | 0.08(0.01-0.61) |
| <b>Spearman’s rank correlation, r (p value) with 90 minute serum C peptide in MMTT</b>       | 0.91 (p<0.0001)   | 0.95 (p<0.0001)  | 0.89 (p<0.0001) |
| <b>Regression equation UCPCR cut offs for serum C-peptide <math>\geq 0.2</math>nmol/mmol</b> | 0.15              | 0.50             | 0.36            |
| <b>Sensitivity/specificity (%)</b>   | 86/100            | 95/100           | 82/97           |

\* 120minute MMTT UCPCR v home evening meal, p<0.0001

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**Supplementary Table 5. UCPCR measurements and correlations with 90minute serum C-peptide during a MMTT, in pediatric patients with Type 1 diabetes (n=21).**

<sup>∞</sup>data available for postprandial evening meal UCPCR, n=15.

|  | UCPCR (nmol/mmol)  |                    |                           |
|--|--------------------|--------------------|---------------------------|
|  | 0 minute           | 120 minute         | Evening meal <sup>∞</sup> |
| <b>UCPCR median(IQR) nmol/mmol</b>   | 0.10(0.03-0.35)    | 0.46(0.11-1.33)*   | 0.38(0.08-0.94)           |
| <b>Spearman's rank correlation, r (p value) with 90 minute serum C peptide in MMTT</b> | 0.92<br>(p<0.0001) | 0.97<br>(p<0.0001) | 0.95<br>(p<0.0001)        |
| <b>Regression equation UCPCR cut offs for serum C-peptide ≥0.2nmol/mmol</b>            | 0.15               | 0.6                | 0.40                      |
| <b>Sensitivity/specificity (%)</b>   | 80/90              | 100/100            | 89/100                    |

\* 120minute MMTT UCPCR v home evening meal, p=0.006

**Supplementary Table 6. UCPCR measurements following 3 home meals and their correlations to 90minute serum C-peptide during a MMTT in 51 adults with Type 1 diabetes.**

*\*Standard breakfast comprised 2 toast, margarine, jam, 250ml orange or apple juice carton.*

|  | UCPCR                           |                     |                     |
|--|---------------------------------|---------------------|---------------------|
|  | 120 minute home meal collection |                     |                     |
|  | Home meal                       | Standard breakfast* | Lunch               |
| <b>UCPCR median (IQR) nmol/mmol</b>                                  | 0.06<br>(0.01-0.43)             | 0.06<br>(0.01-0.68) | 0.08<br>(0.01-0.61) |
| <b>Spearman's rank correlation, r p)</b>                             | 0.94<br>(p<0.0001)              | 0.92<br>(p<0.0001)  | 0.89<br>(p<0.0001)  |
| <b>Equivalent UCPCR cut-off for serum C-peptide &gt;0.2nmol/mmol</b> | 0.21                            | 0.34                | 0.36                |
| <b>Sensitivity/specificity (%)</b>                                   | 86/96                           | 86/97               | 82/97               |