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Research: Treatment

The association between postprandial urinary C-peptide creatinine ratio and the treatment response to liraglutide: a multi-centre observational study

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Abstract

Aims The response to glucagon-like peptide 1 receptor agonist treatment may be influenced by endogenous β-cell function. We investigated whether urinary C-peptide creatinine ratio assessed before or during liraglutide treatment was associated with treatment response.

Methods A single, outpatient urine sample for urinary C-peptide creatinine ratio was collected 2 h after the largest meal of the day among two separate groups: (1) subjects initiating liraglutide (0.6 → 1.2 mg daily) or (2) subjects already treated with liraglutide for 20–32 weeks. The associations between pretreatment and on-treatment urinary C-peptide creatinine ratio and HbA_{1c} change at 32 weeks were assessed using univariate and multivariate analyses (the ratio was logarithm transformed for multivariate analyses). Changes in HbA_{1c} according to pretreatment urinary C-peptide creatinine ratio quartiles are shown.

Results One hundred and sixteen subjects (70 pretreatment, 46 on treatment) with Type 2 diabetes from 10 diabetes centres were studied. In univariate analyses, neither pretreatment nor on-treatment urinary C-peptide creatinine ratio correlated with HbA_{1c} change (Spearman rank correlation coefficient, $r = -0.17$, $P = 0.17$ and $r = -0.20$, $P = 0.19$, respectively). In multi-linear regression analyses, entering baseline HbA_{1c} and log urinary C-peptide creatinine ratio, pretreatment and on-treatment log urinary C-peptide creatinine ratio became significantly associated with HbA_{1c} change ($P = 0.048$ and $P = 0.040$, respectively). Mean (SD) HbA_{1c} changes from baseline in quartiles 1 to 4 of pretreatment urinary C-peptide creatinine ratio were –3 ± 17 mmol/mol (–0.3 ± 1.6%) ($P = 0.52$), –12 ± 15 mmol/mol (–1.1 ± 1.4%) ($P = 0.003$), –11 ± 13 mmol/mol (–1.0 ± 1.2%) ($P = 0.002$) and –12±17 mmol/mol (–1.1±1.6%) (P=0.016), respectively.

Conclusions Postprandial urinary C-peptide creatinine ratios before and during liraglutide treatment were weakly associated with the glycaemic response to treatment. Low pretreatment urinary C-peptide creatinine ratio may be more useful than higher values by predicting poorer glycaemic response.

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Introduction

Glucagon-like peptide 1 (GLP-1) receptor agonists act by mimicking the gut hormone GLP-1. Pharmacological activation of the GLP-1 receptor leads to an increase in insulin secretion, a reduction in hyperglucagonaemia, slowing of gastric emptying and suppression of appetite [1]. The GLP-1 receptor agonists liraglutide (once daily) and exenatide (twice daily or once weekly) have been shown to improve diabetes control in Type 2 diabetes [2–4]. GLP-1 receptor agonists have the advantage of promoting weight loss and treatment is associated with low hypoglycaemia risk. However, disadvantages include an anecdotal variability in treatment response, frequent gastrointestinal side effects

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What's new?

- The efficacy of glucagon-like peptide 1 receptor agonist treatment may be influenced by underlying β-cell function, but this has not been formally tested or reported.
- Urinary C-peptide creatinine ratio is a measure of endogenous insulin secretion. We report a positive association between postprandial urinary C-peptide creatinine ratio collected before and during liraglutide treatment with the glycaemic response to treatment.
- We discuss issues to be clarified or overcome prior to the practical application of this ratio to predict glucagon-like peptide 1 receptor agonist response.

and a higher cost of drug therapy compared with other treatment options [2–5]. Hence, a clinical or biochemical marker that helps predict GLP-1 receptor agonist treatment response will be of great benefit to clinicians when their use is being considered.

It is suspected that the efficacy of GLP-1 receptor agonists, via its action on insulin stimulation, depends on the presence of adequate endogenous β-cell function. For this reason, the use of GLP-1 receptor agonists is contraindicated in patients with Type 1 diabetes [6,7]. In contrast, treatment with GLP-1 receptor agonists has been shown to improve indices of β-cell function in both animal and human studies [8–10]. There are also reports of improvements in measures of diabetes control in individuals with Type 1 diabetes treated with GLP-1 receptor agonists [11,12]. The influence of β-cell function on the treatment response to GLP-1 receptor agonist is therefore unclear. Regardless, it remains difficult to accurately determine the presence of relative insulin deficiency in patients with Type 2 diabetes in day-to-day practice.

C-peptide is produced by the cleavage of proinsulin to insulin. As compared with insulin, C-peptide in the body is less rapidly degraded and is therefore a more stable marker of insulin secretion. Assays of C-peptide would also not detect exogenous insulin treatment [13]. For several decades, 24-h urinary C-peptide has been explored as an option in assessing β-cell function [14,15]. More recently, single-sample stimulated urinary C-peptide creatinine ratio has been shown to correlate well with serum C-peptide [16]. In addition, both urinary C-peptide creatinine ratio levels stimulated by a home meal and that from a standard mixed meal tolerance test compared well with serum C-peptide levels [17]. The ratio of urinary C-peptide to urine creatinine adjusts for the concentration of urine. This is analogous to the use of the albumin creatinine ratio in the assessment of diabetic nephropathy.

The concept that the efficacy of GLP-1 receptor agonist treatment is dependent on endogenous β-cell function has not been formally tested. We therefore hypothesized that, among subjects treated with liraglutide, urinary C-peptide creatinine ratio will be associated with the glycaemic response to treatment. We also sought to determine if a cut-off urinary C-peptide creatinine ratio level could predict a favourable glycaemic response, and whether there was any effect on weight response. We tested our hypothesis in subjects naive to the GLP-1 receptor agonist liraglutide, but also among subjects already prescribed liraglutide. This arm acted as a control in the event that it was not pretreatment β-cell function, but rather β-cell function achieved with treatment, that mattered when assessing the treatment response to liraglutide.

Subjects and methods

Study setting, design and enrolment of subjects

This was a multi-centre study based among specialist diabetes centres in the UK. The study compared urinary C-peptide creatinine ratio levels of subjects starting or started on liraglutide 1.2-mg treatment with their glycaemic response at 32 weeks of treatment. The study consisted of two arms. The first arm involved subjects providing a single urine sample for urinary C-peptide creatinine ratio within a week before they started liraglutide treatment (pretreatment arm). A second arm involved a different group of subjects providing the urine sample when they were between 20 and 32 weeks of liraglutide treatment (on-treatment arm). The diabetes care of subjects continued routinely under the respective diabetes centres, but with a required follow-up at 20–32 weeks. Diabetes treatment changes were allowed during this period in the pretreatment arm to optimize subjects' diabetes control or avoid hypoglycaemia. Follow-up for the purpose of the study concluded at 32 weeks. Subjects were recruited from February 2011 until December 2011, with the final follow-up visit and data collection occurring in June 2012.

Inclusion criteria were broad and included non-insulin or insulin-treated patients with Type 2 diabetes who ordinarily would have been started on GLP-1 receptor agonist treatment based on local centre practice. Subjects excluded were those under 18 years of age, who had been on liraglutide for more than 32 weeks, or those with estimated glomerular filtration rate (eGFR) < 30 ml min^{-1} 1.73 m^{-2}. Estimated GFR was calculated using the four-point Modification of Diet in Renal Disease (MDRD) formula: 30 849 × (serum creatinine in μmol/l)$^{-1.154}$ × (age)$^{-0.203}$ × (0.742 if female) × (1.212 if black) [18].

The study was approved by the Staffordshire Research Ethics Committee and the respective Research and Development departments of each centre prior to patient recruitment.

Urine sample collection and urinary C-peptide creatinine ratio assay characteristics

Subjects were provided instructions (see also Supporting Information, Appendix S1) on how to collect a one-off urine sample

at home 2 h after their largest meal of the day. Samples were sent to the biochemistry department in Exeter in a prepaid reinforced envelope. Samples were collected in boric acid preservative and are known to be stable at room temperature (21 °C) for 3 days. Samples received more than 3 days after collection were considered old and were not analysed.

C-peptide analysis was undertaken on a routine analyser at the biochemistry department at the Royal Devon and Exeter NHS Foundation Trust, Exeter, UK, using the Roche Diagnostics (Mannheim, Germany) E170 analyser (intra-assay coefficient of variation < 3.3%; inter-assay coefficient of variation < 4.5%, functional sensitivity = 0.003 nmol/l) [19]. The assay is a direct electrochemiluminescence immunoassay for human serum, plasma or urine. It is a two-site immunoassay employing monoclonal antibodies against human C-peptide, calibrated to World Health Organization International Reference Reagent for C-peptide of human insulin for immunoassay (IRR code 84/510). Urine creatinine was analysed on the Roche P800 platform (Roche Diagnostics) using creatinine Jaffé reagent (standardized against isotope dilution mass spectrometry) to obtain a urine C-peptide creatinine ratio (nmol/mmol) (intra- and inter-assay coefficient of variation < 2.3%, functional sensitivity 0.36 mmol/l).

Data handling, statistical analyses and sample size calculation

All investigators were blinded to urinary C-peptide creatinine ratio results. The last HbA_{1c} and weight data prior to 32 weeks of treatment were used for analyses. Only HbA_{1c} and weight data obtained during liraglutide treatment were used. For pretreatment subjects who either subsequently discontinued liraglutide prior to 32 weeks, had missed follow-up or had to start insulin treatment, the last HbA_{1c} and weight data prior to these occurring were used, but with a minimum of 10 weeks after liraglutide treatment. Data from pretreatment and on-treatment arms were analysed separately.

Univariate associations of urinary C-peptide creatinine ratio, and HbA_{1c} change, were assessed using non-parametric and parametric statistical methods, respectively. Urinary C-peptide creatinine ratio was logarithm transformed for multivariate analyses. After checking for significant interactions, we performed three multi-linear regression models of HbA_{1c} change to evaluate the effects of different predictor or confounder variables. Model 1 included variables of log urinary C-peptide creatinine ratio and baseline HbA_{1c}. Model 2 included variables in model 1 and other variables significantly associated with urinary C-peptide creatinine ratio in univariate analyses. Model 3 included variables of model 2 and the variable diabetes treatment reduction. Diabetes treatment reduction was defined as categorical variable (0 or 1) and was present when there was discontinuation of a diabetes treatment class or when there was a reduction of total daily insulin dose by more than 20%. This was an arbitrary definition, but follows that of a protocol-driven 20% reduction of insulin dose in a randomized trial adding exenatide to insulin treatment in Type 2 diabetes [20], and the allowed halving (but not stopping) of sulphonlyurea in a trial adding liraglutide to dual oral therapy [21]. Model 3 was listed separately because of the uncertainty of whether diabetes treatment changes were the cause or effect of observed glycaemic changes. Attempts to identify clinically useful cut-off pretreatment urinary C-peptide creatinine ratio values were conducted by assessing HbA_{1c} change across urinary C-peptide creatinine ratio quartiles and the significance assessed by one-way analysis of variance (ANOVA), and analysis of covariance (ANCOVA) with baseline HbA_{1c} as a covariate. *P*-values of < 0.05 were deemed significant for all analyses. Statistical analyses were performed using Minitab® Release 16 (Minitab Ltd, Coventry, UK).

The estimation of the required sample size for the study was difficult because of the lack of prior urinary C-peptide creatinine ratio data on subjects undergoing GLP-1 receptor agonist therapy. We hypothesized that the association between urinary C-peptide creatinine ratio and HbA_{1c} change will have a correlation coefficient of ≥ 0.3 and it would require an estimated 100 subjects (50 pretreatment and 50 on treatment) for multivariate analyses of six variables associated with HbA_{1c} change to yield an F-value of approximately 4. However, because of an anticipated high rate of lost-to-follow-up and mid-trial exclusions among subjects in the pretreatment arm, a more conservative number of 150 subjects was aimed for.

Results

Baseline data

In total, 156 subjects were recruited from 10 diabetes centres. Forty subjects were excluded from study analyses (Fig. 1). Clinical, demographic and urinary C-peptide creatinine ratio data from 116 subjects (70 providing pretreatment urine and 46 providing on-treatment urine) are shown in Table 1.

Pretreatment and on-treatment log urinary C-peptide creatinine ratios were both significantly lower among subjects with older age (Spearman rank correlation coefficient, $r = -0.25$, $P = 0.041$ and $r = -0.32$, $P = 0.028$, respectively), longer duration of diabetes ($r = -0.37$, $P = 0.002$ and $r = -0.41$, $P = 0.005$, respectively) and on insulin treatment [median (interquartile range) urinary C-peptide creatinine ratio 1.51 (0.85–2.92) nmol/mol vs. 2.81 (1.34–4.93) nmol/mol, $P = 0.027$, and 1.19 (0.89–3.16] nmol/mol vs. 3.71 (1.45–6.27) nmol/mol, $P = 0.011$, respectively]. In addition, pretreatment and on-treatment log urinary C-peptide creatinine ratio both correlated with baseline and 32-week eGFR ($r = 0.27$, $P = 0.025$ and $r = 0.35$, $P = 0.021$, respectively) and inversely with baseline or 32-week serum creatinine ($r = -0.31$, $P = 0.008$ and $r = -0.36$, $P = 0.016$, respectively) (see also Supporting Information, Table S1).

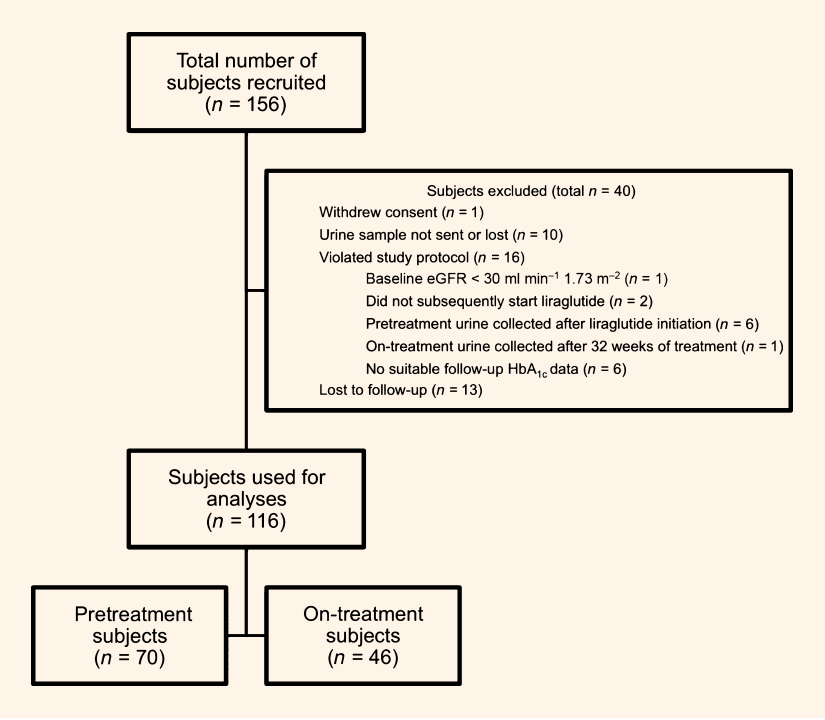

FIGURE 1 Subjects included and excluded in the liraglutide urinary C-peptide creatinine ratio study.

Table 1 Baseline characteristics and urinary C-peptide creatinine ratio values of subjects in the liraglutide urinary C-peptide creatinine ratio study

	Pretreatment	On treatment	Total
n	70	46	116
Age (years), mean ± SD	55 ± 10	60 ± 9	57 ± 10
Male, *n* (%)	31 (44.3)	26 (56.5)	57 (49.1)
Ethnicity, *n* (%)			
Caucasian	58 (82.9)	43 (93.5)	101 (87.1)
South Asian	11 (15.7)	2 (4.3)	13 (11.2)
Afro-Caribbean	1 (1.4)	1 (2.2)	2 (1.7)
Duration of diabetes (years), mean ± SD	10 ± 6	11 ± 6	10 ± 6
Weight (kg), mean ± SD	107.5 ± 21.7	108.7 ± 22.0	108.4 ± 21.4
BMI (kg/m^2), mean ± SD	37.9 ± 5.9	38.5 ± 7.1	38.2 ± 6.4
HbA_{1c} (%), mean ± SD	9.4 ± 1.5	9.2 ± 1.2	9.3 ± 1.4
HbA_{1c} (mmol/mol), mean ± SD	79 ± 16	77 ± 13	78 ± 15
Diabetes treatment, *n* (%)			
1 oral anti-diabetes drug	8 (11.4)	7 (15.2)	15 (12.9)
2 oral anti-diabetes drugs	22 (31.4)	11 (23.9)	33 (28.4)
3 oral anti-diabetes drugs	12 (17.1)	7 (15.2)	19 (16.4)
4 oral anti-diabetes drugs	1 (1.4)	2 (4.3)	3 (2.6)
Basal insulin ± oral anti-diabetes drug	8 (11.4)	9 (19.6)	17 (14.7)
Premixed insulin ± oral anti-diabetes drug	10 (14.2)	8 (17.4)	18 (15.5)
Basal bolus insulin ± oral anti-diabetes drug	9 (12.9)	2 (4.4)	11 (9.5)
Urinary C-peptide creatinine ratio (nmol/mmol)			
Range	< 0.02 to 16.37	0.10 to 14.24	< 0.02 to 16.37
Median (interquartile range)	1.88 (0.96–4.16)	2.37 (1.09–5.24)	2.04 (1.06–4.43)

Effects of treatment

Pretreatment and on-treatment subjects achieved mean (SD) HbA_{1c} change of –0.9% (± 1.5); 10 mmol/mol (± 17) ($P < 0.001$) and –1.4% (± 1.3); 15 mmol/mol (± 14) ($P < 0.001$), both at a median of 24 weeks after liraglutide initiation, respectively. HbA_{1c} change was not associated with subject's age, duration of diabetes, eGFR, baseline weight, BMI, time interval to HbA_{1c} data, gender, ethnicity, the number of background oral anti-diabetes drugs or the presence of background insulin treatment.

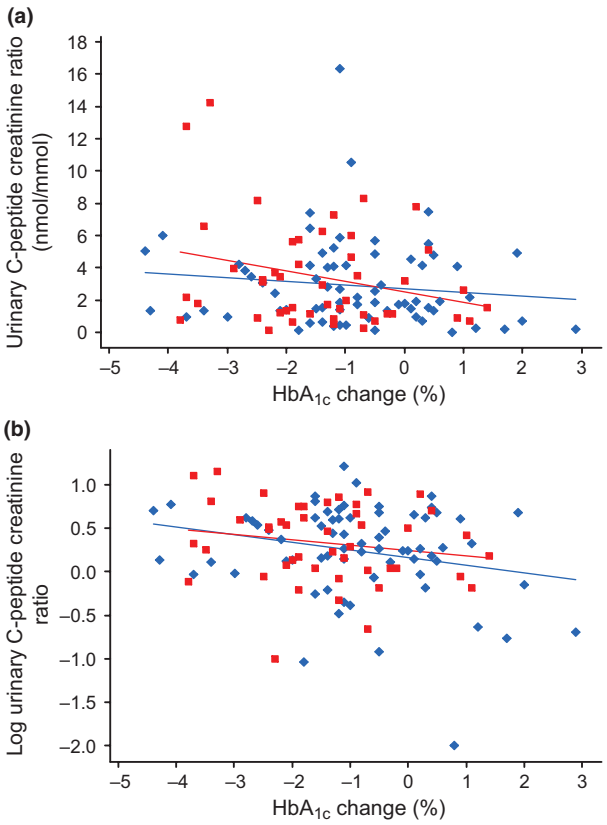

FIGURE 2 Scatterplot to show association between (a) urinary C-peptide creatinine ratio and (b) log urinary C-peptide creatinine ratio and HbA1c change with liraglutide treatment at 32 weeks; (a) pretreatment urinary C-peptide creatinine ratio (◆) (Spearman rank correlation coefficient) $r = -0.17$, $P = 0.17$, on-treatment urinary C-peptide creatinine ratio (■) $r = -0.20$, $P = 0.19$, (b) pretreatment log urinary C-peptide creatinine ratio (◆) (Pearson correlation coefficient) $R = -0.23$, $P = 0.051$, on-treatment log urinary C-peptide creatinine ratio (■) $R = -0.18$, $P = 0.24$. Urinary C-peptide creatinine ratio value of < 0.02 nmol/mmol was taken to be 0.01 for logarithm transformation.

In univariate analyses, neither pretreatment nor on-treatment urinary C-peptide creatinine ratio correlated with HbA_{1c} change (Spearman rank correlation coefficient $r = -0.17$, $P = 0.17$ and $r = -0.20$, $P = 0.19$, respectively). Figure 2 shows these associations in a scatterplot as well as the effect of transforming urinary C-peptide creatinine ratio data into a logarithm scale. Baseline HbA_{1c} was significantly associated with HbA_{1c} change in both subject groups (Pearson correlation coefficient $R = -0.44$, $P < 0.001$ and $R = 0.67$, $P < 0.001$, respectively). In multi-linear regression analyses entering variables of baseline HbA_{1c} and log urinary C-peptide creatinine ratio (model 1), pretreatment and on-treatment log urinary C-peptide creatinine ratios became significantly associated with HbA_{1c} change ($P = 0.048$ and $P = 0.040$). Table 2 shows the effects of adding more variables into the regression models.

Table 3 shows the changes in HbA_{1c} across quartiles of pretreatment urinary C-peptide creatinine ratio and corresponding ranges of urinary C-peptide creatinine ratio values.

Some degree of weight reduction from baseline was achieved by 83.6% of pretreatment subjects and 79.1% of on-treatment subjects. Pretreatment subjects achieved body weight change of mean (SD) –3.4 kg (± 5.0) ($P < 0.001$), or –3.3% (± 4.5) as a proportion of initial body weight, at a median of 26 weeks of treatment. On-treatment subjects achieved body weight change of –3.5 kg (± 4.4) ($P < 0.001$), or –3.3% (± 4.2) of initial body weight at a median of 24 weeks of treatment. Pretreatment and on-treatment percentage weight change were not associated with urinary C-peptide creatinine ratio levels ($r = -0.05$, $P = 0.72$ and $r = 0.03$, $P = 0.85$, respectively).

Sensitivity analyses

Figure 2b suggests that the significant association between pretreatment log urinary C-peptide creatinine ratio and HbA_{1c} change may be driven by the results of subjects with low urinary C-peptide creatinine ratio values. We repeated the multivariate analyses of HbA_{1c} change (model 1) after excluding subjects with pretreatment urinary C-peptide creatinine ratio values that were below the 10th percentile. In this model, pretreatment log urinary C-peptide creatinine ratio was not significantly associated with HbA_{1c} change ($P = 0.58$).

Discussion

Urinary C-peptide is a marker of endogenous insulin secretion and is a convenient and non-invasive outpatient test. Our study prospectively evaluated the association between postprandial urinary C-peptide creatinine ratio levels of subjects before and during liraglutide treatment and their glycaemic response to treatment. Study investigators were blinded to the urinary C-peptide creatinine ratio results to avoid the knowledge of the results potentially influencing the diabetes treatment of subjects. After adjusting for the effects of baseline HbA_{1c}, we found that higher pretreatment and on-treatment urinary C-peptide creatinine ratio levels were associated with greater HbA_{1c} reduction at 32 weeks of treatment.

We had previously reported on the lower glycaemic reduction achieved with exenatide treatment among insulin-treated patients as compared with non-insulin-treated patients [22]. Our unpublished studies suggested a negative influence of diabetes duration on glycaemic outcomes with GLP-1 receptor agonist treatment, although the opposite effect of diabetes duration was found in a different study among patients on basal insulin treated with exenatide [23]. A randomized trial of liraglutide had also shown possible lower HbA_{1c} reduction among patients on a greater number of oral anti-diabetes drugs [24]. We had hypothesized that factors such as number of oral anti-diabetes drugs, need for insulin and diabetes duration were likely imperfect surrogates for the presence of β-cell decline. Indeed, this hypothesis was integral to the study design that allowed subjects on

Table 2 Multi-linear regression analyses of variables associated with HbA_{1c} change in subjects treated with liraglutide

	Model 1		Model 2		Model 3	
	Regression coefficient (95% CI)	*P*-value	Regression coefficient (95% CI)	*P*-value	Regression coefficient (95% CI)	*P*-value
Pretreatment subjects						
Baseline HbA_{1c}	–0.41 (–0.61 to –0.21)	< 0.001	–0.41 (–0.62 to –0.19)	< 0.001	–0.41 (–0.62 to –0.19)	< 0.001
Log urinary C-peptide creatinine ratio	–0.58 (–1.16 to –0.004)	0.048	–0.80 (–1.50 to –0.10)	0.026	–0.73 (–1.46 to –0.01)	0.049
eGFR	—	—	0.002 (–0.014 to 0.018)	0.78	0.000 (–0.016 to 0.017)	0.97
Duration of diabetes	—	—	0.003 (–0.065 to 0.071)	0.93	0.001 (–0.068 to 0.070)	0.98
Age	—	—	–0.004 (–0.037 to 0.028)	0.80	–0.004 (–0.036 to 0.029)	0.82
Insulin-treated	—	—	–0.38 (–1.14 to 0.38)	0.32	–0.31 (–1.10 to 0.49)	0.45
Reduced treatment*	—	—	—	—	0.27 (–0.51 to 1.05)	0.49
On-treatment subjects						
Baseline HbA_{1c}	–0.73 (–0.96 to –0.50)	< 0.001	–0.75 (–0.99 to –0.51)	< 0.001	–0.74 (–0.95 to –0.53)	< 0.001
Log urinary C-peptide creatinine ratio	–0.66 (–1.28 to –0.03)	0.040	–0.23 (–1.04 to 0.58)	0.57	–0.29 (–1.01 to 0.44)	0.43
eGFR	—	—	–0.002 (–0.013 to 0.009)	0.75	–0.005(–0.015 to 0.005)	0.35
Duration of diabetes	—	—	0.02 (–0.04 to 0.07)	0.57	–0.004 (–0.021 to 0.047)	0.89
Age	—	—	0.01 (–0.03 to 0.04)	0.60	0.01 (–0.02 to 0.04)	0.50
Insulin-treated	—	—	0.26 (–0.52 to 1.04)	0.51	0.40 (–0.30 to 1.10)	0.26
Reduced treatment*	—	—	—	—	0.89 (0.35 to 1.44)	0.002

*Treatment reduction was defined as discontinuation of a class of diabetes treatment or reduction of insulin dose by 20% of total daily dose. Regression coefficients with negative values indicate greater HbA_{1c} reduction when there is an increase of the tested variable.

Table 3 HbA_{1c} changes across quartiles of pretreatment urinary C-peptide creatinine ratio

	Q1	Q2	Q3	Q4
n	17	18	18	17
Urinary C-peptide creatinine ratio range (nmol/mmol)	< 0.02–0.94	0.96–1.87	1.89–4.16	4.17–16.37
HbA_{1c} reduction (unadjusted)*				
mmol/mol	–3 ± 17	–12 ± 15	–11 ± 13	–12 ± 17
%	–0.3 ± 1.6	–1.1 ± 1.4	–1.0 ± 1.2	–1.1 ± 1.6
P value	0.52	0.003	0.002	0.016
HbA_{1c} reduction (adjusted)†				
mmol/mol	–5 ± 3	–9 ± 3	–13 ± 3	–11 ± 3
%	–0.5 ± 0.3	–0.8 ± 0.3	–1.2 ± 0.3	–1.0 ± 0.3

HbA_{1c} reduction shown mean (± SD) (unadjusted) and least squares (LS) mean (± SEM) after adjusting for baseline HbA_{1c}.
*$P = 0.27$, †$P = 0.41$ for effect across quartile groups.

various degrees of diabetes treatment to be recruited. We were able to show that urinary C-peptide creatinine ratio levels were lower among subjects with longer duration of diabetes and subjects on insulin treatment, and that urinary C-peptide creatinine ratio levels, rather than these variables that were associated with the subsequent glycaemic response to liraglutide treatment.

Our findings are supported by a study on Japanese subjects that showed postprandial urinary C-peptide levels being associated with successful switching of insulin therapy to liraglutide monotherapy [25].

Our study highlights some important considerations prior to the application of urinary C-peptide creatinine ratio in clinical practice. First, we found that the distribution of urinary C-peptide creatinine ratio normalized significantly after logarithm transformation, similar to other studies reporting on serum C-peptide measures for different purposes [26,27]. Second, urinary C-peptide creatinine ratio levels appeared to be influenced by subjects' underlying renal function. We had excluded subjects with eGFR < 30 ml min^{-1} 1.73 m^{-2} because of the important role of the kidney in the metabolism and excretion of C-peptide. A previous study had shown that urinary C-peptide levels should be interpreted according to gender because of their differences in GFR [28]. (Again, this is analogous to the different cut-off points of urine albumin creatinine ratio for defining microalbuminuria in men and women.) Although not an aim of our study, our data suggest that there was a direct association between urinary C-peptide creatinine ratio and eGFR. The effect of eGFR appeared small in our regression models. Nevertheless, because of a relatively small sample size, it is uncertain whether renal function would

significantly impact on the accuracy of urinary C-peptide creatinine ratio assessment to the degree of influencing its ability to predict GLP-1 receptor agonist treatment response.

Third, the results of our data suggest that the association between pretreatment log urinary C-peptide creatinine ratio and HbA_{1c} change was contributed mainly by subjects with low urinary C-peptide creatinine ratio values achieving poorer glycaemic response. This is supported by the analysis of HbA_{1c} change according to urinary C-peptide creatinine ratio quartiles, and also the non-significant association between log urinary C-peptide creatinine ratio with HbA_{1c} change once subjects with very low urinary C-peptide creatinine ratio were excluded. The clinical implication of this finding may be that low urinary C-peptide creatinine ratio may be a more useful measure to predict poor glycaemic response (to 'rule out' patients from starting treatment) rather than higher urinary C-peptide creatinine ratio values being used as a 'rule-in' measure.

A limitation of our study was the absence of an assessment of concurrent insulin sensitivity to aid interpretation of the urinary C-peptide creatinine ratio data. This would have helped explain whether high levels of insulin secretion (as represented by urinary C-peptide creatinine ratio) represented significant underlying insulin resistance rather than the presence of healthy endogenous β-cell function. More comprehensive tests, such as a hyperinsulinaemic euglycaemic glucose clamp study combined with an arginine test, or an intravenous glucose tolerance test, are able to assess both insulin secretion and insulin sensitivity in individuals. However, these tests are invasive and are technically too demanding for routine use in clinical practice. Simple indices for insulin sensitivity such as the homeostasis model assessment (HOMA) or Quantitative Insulin Sensitivity Check Index (QUICKI) have limited accuracy [29]. QUICKI and the more common HOMA assessments also utilize serum insulin levels rather than C-peptide and are therefore difficult to use in insulin-treated individuals. Because of the difficulties of these methods, we investigated the use of urinary C-peptide creatinine ratio as an accessible stand-alone measure in clinical practice. Another limitation of this observational study is that the changes to diabetes treatments or the differences in aggressiveness of glycaemic optimization in participating centres may have influenced the degree of glycaemic response of subjects. We attempted to reduce this effect by statistically adjusting for diabetes treatment changes in our analyses and by involving a large number of centres.

Further research may be indicated based on our findings. It would be useful to confirm our findings using serum C-peptide or with the use of other GLP-1 receptor agonists. It would also be worthwhile investigating whether the accuracy of urinary C-peptide creatinine ratio can be improved further by incorporating an assessment of insulin sensitivity. However, this would negate the advantage of a single outpatient test.

In conclusion, postprandial urinary C-peptide creatinine ratio levels prior to liraglutide initiation and levels achieved after treatment in subjects with Type 2 diabetes were associated with the glycaemic response to therapy. The efficacy of liraglutide is thus likely influenced by an individual's β-cell function. The strength of this association and the influence of renal function and insulin sensitivity may need to be considered prior to a practical application of urinary C-peptide creatinine ratio for the purpose of predicting glycaemic response to GLP-1 receptor agonist therapy.

Contributors to the liraglutide urinary C-peptide creatinine ratio study

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. The association between pretreatment/on-treatment urinary C-peptide creatinine ratio levels and corresponding baseline/32-week variables.
Appendix S1. Urinary C-peptide creatinine ratio and treatment response to liraglutide study: instructions.