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Postprandial responses of incretin and pancreatic hormones in non-diabetic patients with end-stage renal disease

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ORIGINAL ARTICLE

ABSTRACT

Background. Patients with end-stage renal disease (ESRD) have glucometabolic disturbances resulting in a high prevalence of prediabetes. The underlying pathophysiology remains unclear, but may prove important for the strategies employed to prevent progression to overt diabetes. Meal-induced release of the insulinotropic gut-derived incretin hormones and pancreatic hormones play a critical role in the maintenance of a normal postprandial glucose tolerance.

Methods. We studied patients with ESRD and either normal ($n = 10$) or impaired ($n = 10$) glucose tolerance, and control subjects ($n = 11$). Plasma concentrations of glucose, insulin, glucagon, glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and paracetamol were measured repeatedly during a standardized 4-h liquid meal including 1.5 g paracetamol (added for evaluation of gastric emptying).

Results. Fasting glucose and postprandial glucose responses were comparable between groups ($P > 0.082$). Patients with ESRD exhibited higher fasting levels of GIP and glucagon compared with controls ($P < 0.001$). Baseline-corrected GLP-1 and glucagon responses were enhanced ($P < 0.002$), baseline-corrected insulin responses and insulin excursions were reduced ($P < 0.035$), and paracetamol excursions were delayed ($P < 0.024$) in patients with ESRD compared with controls. None of the variables differed between the two ESRD subgroups.

Conclusions. Non-diabetic patients with ESRD were characterized by reduced postprandial insulin responses despite increased secretion of the insulinotropic incretin hormone GLP-1. Fasting levels and baseline-corrected responses of glucagon were elevated and gastric emptying was delayed in the ESRD patients. These perturbations seem to be caused by uraemia *per se* and may contribute to the disturbed glucose metabolism in ESRD patients.

INTRODUCTION

Non-diabetic patients with end-stage renal disease (ESRD) are characterized by metabolic dysregulation, including impairments in the glucose metabolism. These patients exhibit pathophysiological traits similar to those of diabetic subjects with a normal kidney function, including peripheral insulin resistance, impairments in the incretin system and hyperglucagonaemia [1–5]. The underlying pathophysiology is incompletely understood and the postprandial responses of glucoregulatory gastrointestinal and pancreatic hormones have not been comprehensively investigated.

In recent years, mounting evidence has established the digestive tract as an important factor for the maintenance of a normal postprandial glucose tolerance [6]. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones secreted from

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enteroendocrine L and K cells, respectively. The secretion of GLP-1 and GIP is stimulated by the intraluminal presence of digested nutrients (carbohydrates, fat and protein) in the gut [7–10] and both hormones possess strong glucose-dependent insulinotropic properties almost immediately after meal intake via binding to specific receptors on the pancreatic beta cells [11–13]. The incretin hormones are responsible for up to 70% of the insulin response following ingestion of glucose (the so-called incretin effect) in healthy individuals [14]. Patients with type 2 diabetes, on the other hand, have impairments in the incretin system and furthermore they are characterized by disturbances in the glucagon secretion [9, 15–17]. In a recent study, we demonstrated that non-diabetic patients with ESRD had a reduced incretin effect and severely elevated plasma glucagon levels that were non-suppressible after glucose administration, thus similar to patients with type 2 diabetes [5].

A mixed meal test is a strong stimulus for the secretion of incretin hormones and is, therefore, suitable for detecting potential differences in nutrient-stimulated incretin responses [7, 8, 10, 18]. Thus, in the present study, we aimed to further delineate the postprandial secretory responses of incretin hormones and pancreatic hormones in non-diabetic patients with ESRD during a high-calorie mixed meal.

MATERIALS AND METHODS

Study protocol

The study protocol was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (H-C-2009-007) and by the Danish Data Protection Agency (2007-58-0015) and was registered with ClinicalTrials.gov (NCT01327378). A written informed consent was obtained from all participants before inclusion, and the study was conducted according to the latest revision of the Helsinki Declaration.

Subjects

We included 20 patients with ESRD and 11 control subjects, predominantly Caucasians. The subjects also participated in another study, which was recently published along with detailed subject characteristics [5]. In short, patients were divided into two subgroups according to glucose tolerance (evaluated by World Health Organization criteria [19]): normal glucose tolerance (NGT) or impaired glucose tolerance (IGT), following a 2-h 75-g glucose tolerance test (OGTT) performed at an initial screening day. Each subgroup comprised 10 patients. Patients as one group ($n = 20$) were matched with control subjects according to age, gender and body mass index (BMI). The two dialysis subgroups were not mutually matched. Patients fulfilled the following inclusion criteria: age between 18 and 90 years, chronic haemodialysis treatment (through minimum 3 months) and BMI 18.5–28.0 kg/m^2. Patients with diabetes, impaired fasting glucose, first-degree relatives with diabetes, pancreatitis (chronic or acute), previous bowel resection, inflammatory bowel disease, malignancy (previous or actual), daily intake of medication known to influence glucose metabolism (including oral glucocorticoids, thiazides, cyclosporine, etc.) or severe anaemia were excluded. Control subjects had NGT and fulfilled the same exclusion and inclusion criteria apart from a normal kidney function (plasma creatinine <105 μmol/L for men and <90 μmol/L for women). No participants had clinically significant cardiovascular disease or other significant somatic diseases, except for well-treated, mild-to-moderate hypertension. Detailed subject characteristics are presented in Table 1.

Experimental procedures

The meal test was performed after a minimum of 72 h from the screening day. Participants were examined in a recumbent position in the morning after an overnight fast (10 h). No alcohol consumption or vigorous physical activities were permitted 24 h prior to examination and patients were free of dialysis treatment for a minimum of 36 h before the meal test. A cannula was inserted into a dorsal hand vein (opposite any arterio-venous fistula). The cannulated hand was wrapped in a heat pad (50°C) for arterialization of the blood. Each subject ingested 100 g of NAN 1® (Nestle Nordic, Copenhagen, Denmark) [2200 KJ (520 kcal): 58 g carbohydrate, 27.7 g fat and 9.5 g protein] and 1500 mg pulverized paracetamol dissolved in 300 mL water over 10 min (0–10 min). Blood samples were drawn repeatedly at time −15, −10, 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180 and 240 min, and distributed into chilled tubes containing EDTA plus a specific dipeptidyl peptidase 4 inhibitor (valine-pyrrolidide, final concentration = 0.01 mmol/L) for plasma analyses of GIP, GLP-1 and glucagon. Plasma glucose was determined bedside, following collection of blood into *pico tubes*. For analysis of insulin, blood was distributed into dry tubes for coagulation (20 min at room temperature) and blood for paracetamol analysis was collected in chilled tubes containing heparin. All chilled tubes were immediately cooled on ice and centrifuged (1200 g for 20 min at 4°C). Plasma samples were stored at −80°C until analysis.

Analyses

Plasma glucose concentrations were measured during the experiments using an ABL800 FLEX (Radiometer Medical ApS, Brønshøj, Denmark). All other samples were measured *en bloc* following study termination. Plasma insulin concentrations were measured using enzyme-linked immunosorbent assay kits (Elecsys, Roche Diagnostics GmbH, Mannheim, Germany) and the serum paracetamol concentration was measured by the Vitros ACET slide method based on an acryl acid amidase reaction linked to a colour shift reaction using liquid chromatography for quantification as described elsewhere [20]. All assays were automated and performed on a Cobas Fara robot (Roche Diagnostics GmbH, Mannheim, Germany). Plasma samples were assayed for total GLP-1 immunoreactivity using an antiserum that reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite. Total GIP was measured using the C-terminally directed antiserum 867, which reacts fully with intact GIP and the N-terminally truncated metabolite [21]. The glucagon assay was directed against the C-terminal of the glucagon molecule and, therefore, measured glucagon of pancreatic origin. Glucagon analyses were performed as previously described [22].

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Table 1. Clinical and demographical data

	ESRD + NGT	ESRD + IGT	ESRD+NGT + ESRD+IGT	Control
Number (*n*)	10	10	20	11
Age (years)	40.9 ± 2.6$^{\$}$	56.4 ± 5.0	48.7 ± 3.3	47.7 ± 3.8
Gender (m/f)	6/4	8/2	14/6	7/4
BMI (kg/m^2)	21.2 ± 0.9$^{\$}$	24.5 ± 1.0	22.8 ± 0.7	22.2 ± 0.8
Caucasian (*n*)	8	8	16	11
Renal				
Dialysis duration (months)	61 ± 19	37 ± 13	49 ± 11	
Diuresis (mL/day)	704 ± 266	912 ± 331	814 ± 210	
Dialysis adequacy (Kt/V week)	5.0 ± 0.4	4.8 ± 0.5	4.9 ± 0.3	
Co-morbidity				
Hypertension (%)	60	80$^{¤¤}$	70**	9†
Clinical				
Systolic blood pressure (mmHg)	132 ± 4$^{\$\$}$	163 ± 5$^{¤¤¤}$	147 ± 5*	123 ± 6
Diastolic blood pressure (mmHg)	83 ± 2$^{\$}$	95 ± 3$^{¤¤}$	89 ± 2*	76 ± 4
Laboratory results—screening				
Haemoglobin (mmol/L)	7.4 (7.0–7.9)	7.5 (7.0–8.0)$^{¤¤}$	7.4 (7.1–7.8)***	8.8 (8.4–9.1)††
Creatinine (µmol/L)	793 (691–910)	776 (634–950)$^{¤¤¤}$	784 (701–877)***	66 (59–73)†††
Urea nitrogen (mmol/lL	15.1 (13.1–17.4)	16.3 (12.9–20.7)$^{¤¤¤}$	15.7 (13.9–17.9)***	4.8 (4.1–5.7)†††
Sodium (mmol/L)	141 (137–145)	141 (139–143)	141 (139–143)	142 (141–143)
Potassium (mmol/L)	4.5 (4.0–5.1)	4.6 (4.2–5.0)$^{¤¤}$	4.6 (4.3–4.8)***	3.7 (3.5–3.8) †
Albumin (g/L)	38 (32–46)	41 (38–45)	40 (36–44)*	46 (44– 48)†
Total carbon dioxide (mmol/L)	27 (24–30)	26 (24–29)	27 (25–29)	27 (25–29)
C-reactive protein (mg/l)	2.0 (0.8–4.7)	5.1 (2.6–10.0)$^{¤}$	3.3 (1.9–5.6)*	1.1 (0.4–1.4)
Haemoglobin A1c (%)	4.9 (4.7–5.2)	5.1 (4.7–5.6)	5.0 (4.8–5.3)	5.3 (5.1–5.4)
HOMA-IR (index)	1.11 ± 0.12	1.47 ± 0.20	1.29 ± 0.12	1.33 ± 0.12
Fasting laboratory results—meal test				
Glucose (mmol/L)	5.2 (4.8–6.5)	5.3 (5.0–5.6)	5.2 (5.0–5.5)	5.3 (5.1–5.6)
Insulin (pmol/L)	35.9 (25.4–50.6)	30.1 (23.0–39.3)	32.9 (26.9–40.2)	31.5 (25.3–39.3)
Glucagon (pmol/L)	19.1 (14.7–24.9)	18.5 (13.6–25.1)$^{¤¤¤}$	18.8 (15.7–22.6)***	5.2 (3.8–7.1)†††
Total GLP-1 (pmol/L)	9.0 (6.0–13.4)	10.3 (7.2–14.6)	9.6 (7.6–12.2)	6.8 (5.0–9.4)
Total GIP (pmol/L)	16.2 (12.0–21.8)	16.4 (9.3–29.1)$^{¤}$	16.3 (12.2–21.8)**	7.3 (5.3–10.0)†
Paracetamol (mmol/L)	0.00	0.00	0.00	0.00

BMI, body mass index; ESRD, end-stage renal disease; HOMA-IR, homeostasis model assessment of insulin resistance; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; IGT, impaired glucose tolerance; K_t/V, clearance × time/volume (dimensionless); NGT, normal glucose tolerance.
Data are presented as means ± SEM, geometric means with 95% confidence intervals, numbers (*n*) or percent (%). Fasting concentrations before the meal test were calculated as mean of −15, −10 and 0 min samples. One-way ANOVA with *post hoc* analyses (Tukey's HSD test) or unpaired *t* test was used where appropriate.
ESRD+NGT + ESRD+IGT versus control: *P < 0.05, **P < 0.001, ***P < 0.0001; ESRD+NGT versus control: †P < 0.05, ††P < 0.001, †††P < 0.0001; ESRD+NGT versus ESRD+IGT: $^{\$}$P < 0.05, $^{\$\$}$P < 0.001, $^{\$\$\$}$P < 0.0001; ESRD+IGT versus control: $^{¤}$P < 0.05, $^{¤¤}$P < 0.001, $^{¤¤¤}$P < 0.0001.

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Calculations and statistical analyses

Results are expressed as means ± SEM or geometric means with 95% confidence intervals unless otherwise stated. Fasting levels of plasma glucose, insulin, glucagon, GLP-1 and GIP were evaluated from the mean of −15 min, −10 min and 0 min values before the meal test. Area under the curve (AUC) was calculated using the trapezoidal rule and used as a measure of total responses (tAUC) and baseline-corrected responses [incremental AUC (iAUC)]. Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA-IR) and calculated as [fasting plasma insulin (μUnits/mL) × fasting plasma glucose (mmol/L)]/22.5 [23] from data obtained during the OGTT at the screening day. A repeated measures ANOVA model was used to assess curve excursions of the different parameters. Time and group interaction was used to test for equal relative excursions of the repeated measurements during the meal test. Comparisons between groups were performed with one-way ANOVA using Tukey's HSD *post hoc* test (more than two groups) or unpaired *t* test (two groups). In regard to all biomarkers, the statistical analyses were performed on logarithmically transformed data. Subsequently, data were back-transformed and presented as geometric means with 95% confidence intervals. The remaining parameters were analysed and presented as means ± SEM. To evaluate the influence of insulin resistance on the hormone responses, comparisons between groups were done using a univariate general linear model with incremental responses of glucose, insulin, glucagon, GLP-1 and GIP as individual-dependent variables and HOMA-IR as covariate. All tests were two-tailed and a P value of <0.05 was considered significant.

RESULTS

Glucose

Fasting plasma glucose values were comparable (P > 0.702) and within the normal range [19] in the two ESRD subgroups and the control group (Table 1). Glycaemic excursions were limited in all three groups, consistent with the non-diabetic state and the composition of the meal [24]. Both ESRD subgroups had delayed and prolonged glucose excursions with reduced peak values (P < 0.026; Figure 1a–c), whereas incremental (baseline-corrected) responses were similar between all three groups (P > 0.082; Table 2). None of the glucose variables differed significantly between the two ESRD subgroups, although ESRD patients with IGT showed a tendency towards increased incremental responses (P > 0.082).

Insulin and glucagon

Fasting insulin levels did not differ between groups (P > 0.582; Table 1). Insulin excursions, however, were lower in both ESRD subgroups, including reduced peak values (P < 0.009; Figure 2a–c). Likewise, incremental insulin responses were decreased in all ESRD patients compared with control subjects (P = 0.035; Table 2). Fasting concentrations of glucagon were markedly increased in the ESRD patients compared with control subjects (P < 0.001; Table 1). Both ESRD subgroups exhibited net increases during the meal test, which was significantly different from the control group, who exhibited net suppression during the test (P < 0.038; Figure 2d–f and Table 2). No significant differences in any of the insulin or glucagon variables were observed between the two ESRD subgroups.

GLP-1 and GIP

ESRD patients as one group exhibited higher fasting levels of GLP-1 and GIP than the controls (P = 0.073 and 0.001, respectively; Table 1). Both ESRD subgroups had higher peak concentrations and increased excursions of GLP-1 (P < 0.035; Figure 3a–c), while GIP excursions were similar for ESRD patients and controls (P = 0.124; Figure 3d–f). Likewise, the incremental GLP-1 response was higher in ESRD patients than in controls (P = 0.002; Table 2), whereas incremental GIP responses were similar for ESRD patients and controls (P = 0.791; Table 2). No significant differences of GLP-1 or GIP variables were observed between the two ESRD subgroups.

Paracetamol

Evaluation of the curve excursions revealed a slower increase and delayed peak values in both ESRD subgroups compared with the controls (P < 0.024; Figure 4). Incremental responses were reduced, although non-significant, in the ESRD patients compared with controls (P = 0.051; Table 2). There were no significant differences between the two ESRD subgroups.

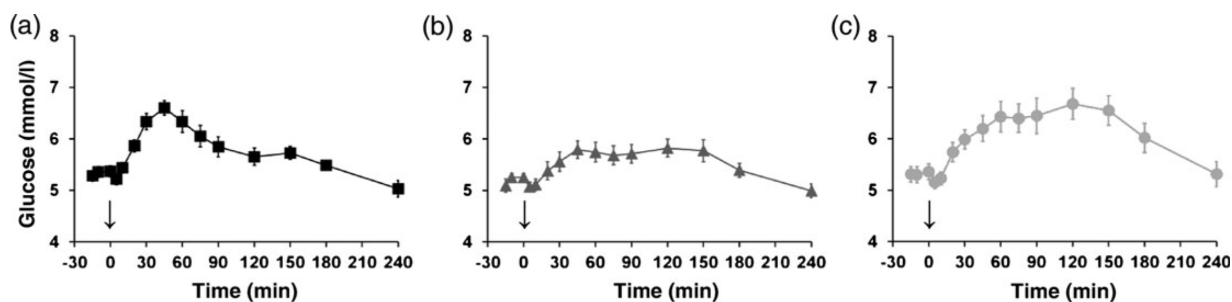

FIGURE 1: Glucose. Plasma glucose in control subjects (**a**) and in patients with ESRD and normal glucose tolerance (**b**) or impaired glucose tolerance (**c**) during a mixed meal test. Data are expressed as means ± SEM. Arrows (↓) indicate the time for initiation of meal intake.

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Table 2. Total and incremental responses				
	ESRD + NGT	ESRD + IGT	ESRD+NGT + ESRD+IGT	Control
Number (*n*)	10	10	20	11
Plasma glucose				
tAUC (min × mmol/L)	1327 ± 30[$]	1468 ± 52	1397 ± 33	1373 ± 17
iAUC (min × mmol/L)	79 ± 33	191 ± 49	135 ± 31	93 ± 17
Plasma insulin				
tAUC (min × nmol/L)	22.9 ± 2.8	21.2 ± 2.0	22.0 ± 1.7	26.1 ± 2.1
iAUC (min × nmol/L)	13.4 ± 2.0	13.6 ± 1.6	13.5 ± 1.2*	18.2 ± 1.7
Plasma glucagon				
tAUC (min × pmol/L)	5554 ± 456	5444 ± 789[¤¤¤]	5499 ± 444***	1240 ± 126[†††]
iAUC (min × pmol/L)	714 ± 179	628 ± 302[¤]	671 ± 171**	−127 ± 109[†]
Total GLP-1				
tAUC (min × nmol/L)	5.5 ± 0.5	6.5 ± 0.7[¤]	6.0 ± 0.4**	3.6 ± 0.4[†]
iAUC (min × nmol/L)	3.0 ± 0.4	3.7 ± 0.6[¤]	3.4 ± 0.4*	1.8 ± 0.3
Total GIP				
tAUC (min × nmol/L)	14.2 ± 1.5	18.6 ± 2.4	16.4 ± 2.0	14.3 ± 1.7
iAUC (min × nmol/L)	10.1 ± 1.2	13.6 ± 1.8	11.8 ± 1.5	12.4 ± 1.8
Paracetamol				
AUC (min × pmol/L)	10.7 ± 2.1	10.4 ± 2.0	10.5 ± 1.4	15.7 ± 2.0

ESRD, end-stage renal disease; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; iAUC, incremental area under curve; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; tAUC, total area under curve.
Data are presented as means ± SEM. One-way ANOVA with *post hoc* analyses (Tukey's HSD test) or unpaired *t* test was used where appropriate. ESRD+NGT + ESRD+IGT versus control: *P < 0.05, **P < 0.001, ***P < 0.0001; ESRD+NGT versus control: [†]P < 0.05, [†††]P < 0.0001; ESRD+NGT versus ESRD+IGT: [$]P < 0.05; ESRD+IGT versus control: [¤]P < 0.05, [¤¤¤]P < 0.0001.

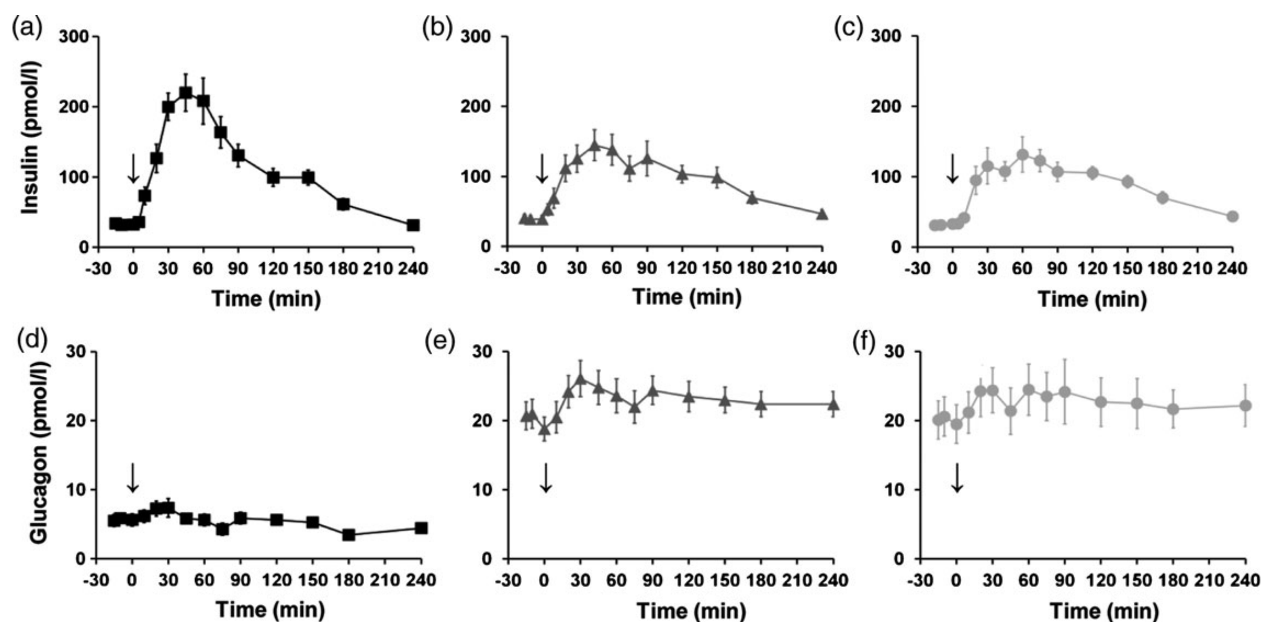

FIGURE 2: Insulin and glucagon. Plasma insulin (**a**–**c**) and glucagon concentrations (**d**–**f**) in control subjects (a and d) and in patients with ESRD and normal glucose tolerance (b and e) or impaired glucose tolerance (c and f), during a mixed meal test. Data are expressed as means ± SEM. Arrows (↓) indicate the time of initiation of meal intake.

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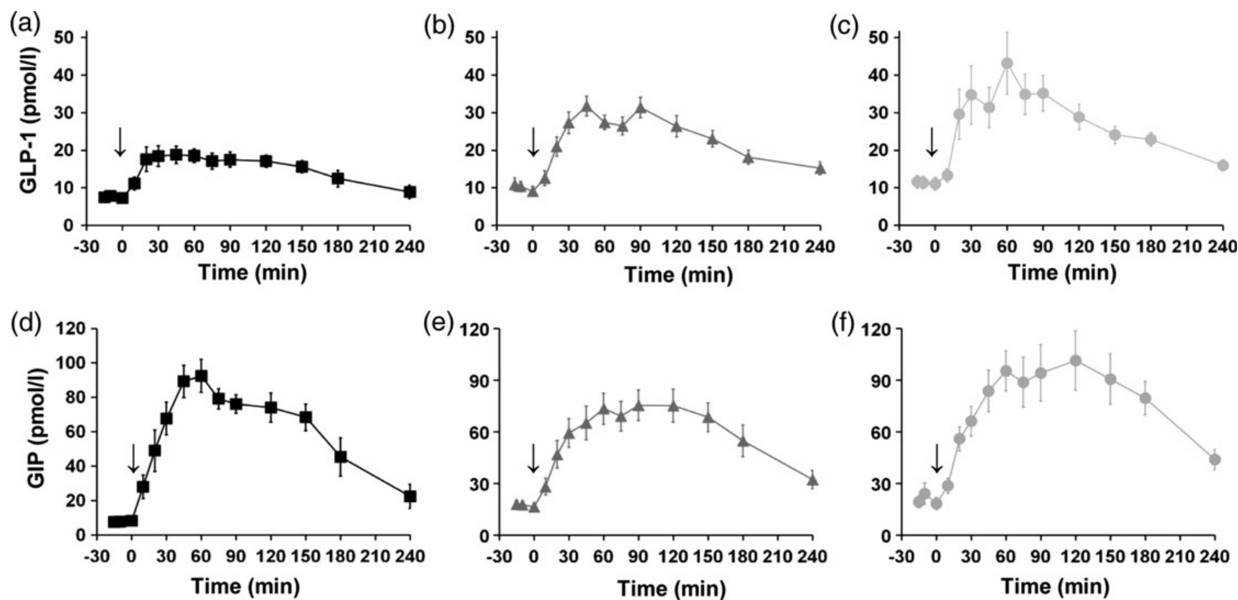

FIGURE 3: Total GLP-1 and GIP. Plasma total GLP-1 responses (**a**–**c**) and total GIP responses (**d**–**f**) in control subjects (a and d) and in patients with ESRD and normal glucose tolerance (b and e) or impaired glucose tolerance (c and f) during a mixed meal test. Data are means ± SEM. Arrows (↓) indicate the time of initiation of meal ingestion. GLP-1: Glucagon-like peptide-1. GIP: Glucose-dependent insulinotropic polypeptide.

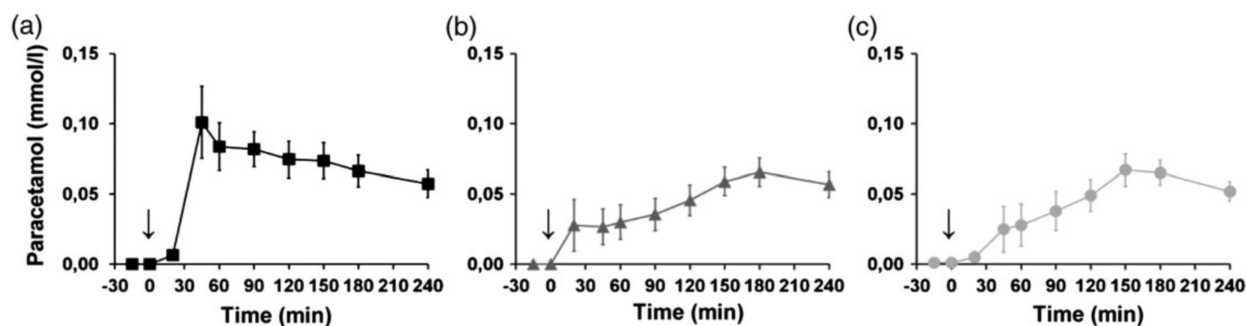

FIGURE 4: Paracetamol. Plasma paracetamol concentrations in control subjects (**a**) and in patients with ESRD and normal glucose tolerance (**b**) or impaired glucose tolerance (**c**). Data are expressed as means ± SEM. Arrows (↓) indicate the time of intake of meal with paracetamol.

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Insulin resistance

Insulin resistance was evaluated from HOMA-IR [23]. HOMA-IR was low [25] in all groups and there were no differences between patients and controls (P > 0.23). Adjustment of the incremental responses of glucose, insulin, glucagon, GLP-1 and GIP for HOMA-IR did not change the results (data not shown).

DISCUSSION

In the present study, we show that non-diabetic ESRD patients are characterized by (i) reduced insulin secretory responses, (ii) increased postprandial GLP-1 responses, (iii) fasting hyperglucagonaemia and excessive glucagon secretion and (iv) delayed gastric emptying for a liquid mixed meal.

Progression of non-diabetic chronic kidney disease is associated with metabolic dysregulation including impairments in the glucose metabolism and an increased risk of prediabetes [1, 3, 4]. Since both uraemia and prediabetes may contribute to these glucometabolic disturbances individually, we investigated patients with ESRD and either NGT or prediabetes (IGT). This enabled us to evaluate the separate impacts of isolated ESRD and ESRD combined with prediabetes on the secretory responses of incretin hormones and pancreatic hormones following meal ingestion. An oral glucose tolerance test causes excess glycaemic excursions compared with a standardized mixed meal challenge [24]. The magnitude of GLP-1 and GIP secretion (but not glycaemic excursions) is, on the other hand, proportional to the caloric content of meals [18]. Thus, a balanced high-calorie mixed meal represents a strong and potent stimulus for the secretion of incretin hormones [7, 8, 10], which makes it suitable for detecting potential differences in nutrient-stimulated incretin responses.

Despite identical glucose responses to a mixed meal in patients with ESRD and control subjects, the concomitant secretory responses and curve excursions of gut-incretin

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hormones and pancreatic hormones differed between groups in our study. The insulin response was significantly reduced in ESRD patients compared with controls despite a significantly increased baseline-corrected GLP-1 response and comparable GIP responses between groups. This suggests that the beta cells in non-diabetic patients with ESRD are unable to respond adequately to GLP-1 and GIP; with the secretion of GLP-1 being enhanced as a potential compensatory mechanism. These findings are in line with and expand the results of a recent study by our group. In the same cohort of participants, we found a reduced incretin effect despite normal or slightly increased incretin hormone responses to oral glucose stimulation, which is also indicative of a reduced incretin-mediated insulin response from the beta cells [5]. In the present study, the differences in the secretory response of GLP-1 between groups were more pronounced and significant, most obviously explained by a stronger stimulation of the enteroendocrine cells during a high-caloric mixed meal compared with pure glucose stimulation. One study has previously examined the postprandial secretory responses of incretins and insulin in patients with ESRD. Miyamoto *et al.* studied 9 haemodialysis patients and 10 healthy controls following synchronous ingestion of a high-fat liquid mixed meal and 75 g glucose dissolved in 500 mL water. The results revealed significant fasting hyperinsulinaemia in the dialysis group, but comparable incremental insulin responses between groups. Fasting GLP-1 and total GLP-1 responses were similar between groups, whereas both fasting and total responses of GIP were increased in the dialysis group [26]. The results differ from our data and a number of explanations for this are evident: Miyamoto *et al.* did not perform an initial oral glucose tolerance test and patients with IGT or overt diabetes, but normal fasting glucose, may therefore have been included. Furthermore, examinations were performed the day after dialysis treatment, that is, not in a maximal uraemic state. Also, the investigators did not evaluate gastric emptying, which could potentially be affected by the high-volume and high-fat meal. Finally, none of the parameters measured in the study reached baseline levels after 4 h of follow-up due to the excessive meal stimulus. These differences may at least to some extent explain the discrepancies observed between the two studies.

Patients with type 2 diabetes or prediabetes, and normal kidney function are characterized by fasting and postprandial hyperglucagonaemia. These perturbations contribute importantly to the hyperglycaemic state of type 2 diabetes [15–17]. In the present study, we confirmed the presence of high fasting glucagon levels in ESRD patients [2, 5, 27]. Moreover, we made a novel observation of excessive glucagon secretion in the ESRD group during mixed meal stimulation, which was significantly different from the response observed in the control group. Again, these results correlate well with and expand the findings from our previous study; following both oral and intravenous glucose stimulation all non-diabetic ESRD patients exhibited impaired glucagon suppression [5]. While a negligible glucagon secretory response is usually observed in healthy individuals following a mixed meal [28], pure protein stimulation results in a marked glucagon secretory response [29, 30]. To our knowledge, glucagon responses to mixed meals have never been examined in ESRD patients, although two groups have previously examined glucagon responses to protein stimulation in patients with impaired kidney function. Bilbrey *et al.* measured glucagon responses after a solid beef meal (1 g lean beef/kg body weight) and found the secretory response to be significantly higher in six uraemic patients (dialysis and non-dialysis) compared with seven control subjects [2, 16]. Sherwin *et al.* examined glucagon responses for 90 min following intravenous infusion of L-alanine (0.15 g/kg body weight) over 2–4 min in 9 uraemic subjects (dialysis and non-dialysis) and in 21 controls. The incremental glucagon response was significantly higher and more prolonged in the uraemic group. However, both groups reached identical baseline-corrected levels after 90 min, indicative of a preserved ability to degrade and eliminate glucagon [27]. Although not directly comparable, the results of the two studies referred to above are in accordance with our findings; uraemic patients seem to have exaggerated glucagon secretion during meal stimulation, irrespective of glucose tolerance status and irrespective of concomitant glucose levels. Similar findings have been shown in diabetic subjects with normal kidney function [15–17]. This further supports the hypothesis that reduced renal catabolism cannot alone explain these abnormal glucagon results, and that concurrent activity of a stimulating factor of the alpha cell (e.g. GIP [31]) overriding the physiological counter-suppression (normally exerted by glucose, GLP-1 and insulin) and resulting in hypersecretion is a likely explanation, although further studies are needed to clarify this. Taken together, the combination of severe fasting hyperglucagonaemia and excessive secretion of glucagon during mixed meal stimulation observed in the present study suggests that glucagon plays a role in the disturbed glucose metabolism in non-diabetic ESRD patients.

We used the paracetamol absorption test to evaluate gastric emptying [32]. Our data indicate a reduced gastric emptying rate in the ESRD patients, which has previously been shown [33], while others have found normal emptying rates in patients with ESRD [34]. Our results are interesting, given the concomitantly increased GLP-1 response. GLP-1 is known to reduce gastric emptying [35] and, thus, it may be the underlying cause. Also, gastric emptying is known to be a strong determinant of GLP-1 secretion [36]. Therefore, the reduced gastric emptying observed in the present study strengthens the notion of increased postprandial GLP-1 secretion in ESRD patients; perhaps constituting a compensatory mechanism driven by a reduced ability of the beta cell to respond to GLP-1.

Our study has some limitations. The sample size is limited and the possibility of type 2 error cannot be precluded. Nonetheless, the number of examined ESRD patients ($n = 20$) is above most similar studies within this area. Also, the ESRD patients comprised a heterogeneous group with different underlying renal diseases. To minimize this confounder, we matched patients and controls on age, gender and BMI, and gave preference to ESRD patients with little co-morbidity to be able to evaluate the separate impact of uraemia. Finally, we did not measure the active components of the incretin hormones separately. The inactive metabolites of GLP-1 and GIP have been proposed to be cleared renally, which could potentially

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explain the increased GLP-1 responses in the ESRD groups. Still, fasting GLP-1 concentrations were comparable between groups, GIP responses were similar between groups and the slope of the GLP-1 and GIP curves during declining hormone concentrations (Figure 3a–d, 120–240 min) were comparable between groups, suggesting a preserved ability to degrade and excrete both active components and inactive metabolites.

To summarize, our data suggest that both pancreatic beta cell and alpha cell disturbances may contribute in the pathogenesis of the disturbed glucose metabolism in patients with ESRD. Although the current study was not designed to characterize the beta cell in detail, a mixed meal test has previously been proposed as a valid measure of the beta cell secretory capacity in subjects with normal kidney function [37]. The presence of beta cell defects is detectable in subjects prior to development of overt type 2 diabetes [38] and, therefore, it is possible that our findings represent early glucometabolic disturbances that can still be compensated for. Future studies using clamp techniques during infusion of GLP-1, GIP and various tracers are warranted for a thorough characterization of the alpha cell and beta cell function and for the implication of the highly abnormal glucagon results on endogenous glucose production.

In conclusion, we have demonstrated new aspects of the impaired glucose metabolism in ESRD patients. The secretory response of GLP-1 was increased in non-diabetic patients with ESRD following a liquid mixed meal, although insulin responses and gastric emptying were reduced. Fasting concentrations of GIP and glucagon were enhanced and the glucagon response was excessive. These perturbations suggest that the incretin system and glucagon are involved in the underlying pathogenesis of the disturbed glucose metabolism in non-diabetic ESRD patients. All pathological findings in the ESRD groups were irrespective of glucose tolerance status, suggesting that the disturbances can most likely be explained by severe uraemia *per se*.

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CONFLICT OF INTEREST STATEMENT

None declared. The results presented in this paper have not been published previously in whole or part, except in abstract format.

REFERENCES

1. Alvestrand A, Mujagic M, Wajngot A *et al.* Glucose intolerance in uraemic patients: the relative contributions of impaired beta-cell function and insulin resistance. Clin Nephrol 1989; 31: 175–183
2. Bilbrey GL, Faloona GR, White MG *et al.* Hyperglucagonemia of renal failure. J Clin Invest 1974; 53: 841–847
3. DeFronzo RA. Pathogenesis of glucose intolerance in uraemia. Metabolism 1978; 27: 1866–1880
4. Eldin WS, Ragheb A, Klassen J *et al.* Evidence for increased risk of prediabetes in the uraemic patient. Nephron Clin Pract 2008; 108: c47–c55
5. Idorn T, Knop FK, Jorgensen M *et al.* Gastrointestinal factors contribute to glucometabolic disturbances in nondiabetic patients with end-stage renal disease. Kidney Int 2013; 83: 915–923
6. Vella A, Camilleri M, Rizza RA. The gastrointestinal tract and glucose tolerance. Curr Opin Clin Nutr Metab Care 2004; 7: 479–484
7. Carr RD, Larsen MO, Winzell MS *et al.* Incretin and islet hormonal responses to fat and protein ingestion in healthy men. Am J Physiol Endocrinol Metab 2008; 295: E779–E784
8. Carrel G, Egli L, Tran C *et al.* Contributions of fat and protein to the incretin effect of a mixed meal. Am J Clin Nutr 2011; 94: 997–1003
9. Deacon CF, Ahren B. Physiology of incretins in health and disease. Rev Diabet Stud 2011; 8: 293–306
10. Herrmann C, Goke R, Richter G *et al.* Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion 1995; 56: 117–126
11. Hare KJ, Vilsboll T, Asmar M *et al.* The glucagonostatic and insulinotropic effects of glucagon-like peptide 1 contribute equally to its glucose-lowering action. Diabetes 2010; 59: 1765–1770
12. Deacon CF, Plamboeck A, Rosenkilde MM *et al.* GIP-(3-42) does not antagonize insulinotropic effects of GIP at physiological concentrations. Am J Physiol Endocrinol Metab 2006; 291: E468–E475
13. Nauck MA, Bartels E, Orskov C *et al.* Additive insulinotropic effects of exogenous synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1-(7-36) amide infused at near-physiological insulinotropic hormone and glucose concentrations. J Clin Endocrinol Metab 1993; 76: 912–917
14. Nauck MA, Homberger E, Siegel EG *et al.* Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab 1986; 63: 492–498
15. Henkel E, Menschikowski M, Koehler C *et al.* Impact of glucagon response on postprandial hyperglycemia in men with impaired glucose tolerance and type 2 diabetes mellitus. Metabolism 2005; 54: 1168–1173
16. Muller WA, Faloona GR, guilar-Parada E *et al.* Abnormal alpha-cell function in diabetes. Response to carbohydrate and protein ingestion. N Engl J Med 1970; 283: 109–115
17. Shah P, Basu A, Basu R *et al.* Impact of lack of suppression of glucagon on glucose tolerance in humans. Am J Physiol 1999; 277: E283–E290

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18. Rijkelijkhuizen JM, McQuarrie K, Girman CJ *et al*. Effects of meal size and composition on incretin, alpha-cell, and beta-cell responses. Metabolism 2010; 59: 502–511
19. Alberti KG, Zimmet PZ. Definition, diagnosis classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15: 539–553
20. Miceli JN, Aravind MK, Cohen SN *et al*. Simultaneous measurements of acetaminophen and salicylate in plasma by liquid chromatography. Clin Chem 1979; 25: 1002–1004
21. Lindgren O, Carr RD, Deacon CF *et al*. Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. J Clin Endocrinol Metab 2011; 96: 2519–2524
22. Knop FK, Vilsboll T, Madsbad S *et al*. Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. Diabetologia 2007; 50: 797–805
23. Matthews DR, Hosker JP, Rudenski AS *et al*. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419
24. Wolever TM, Chiasson JL, Csima A *et al*. Variation of postprandial plasma glucose, palatability, and symptoms associated with a standardized mixed test meal versus 75g oral glucose. Diabetes Care 1998; 21: 336–340
25. Ascaso JF, Pardo S, Real JT *et al*. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. Diabetes Care 2003; 26: 3320–3325
26. Miyamoto T, Rashid QA, Yamamoto T *et al*. Postprandial metabolic response to a fat- and carbohydrate-rich meal in patients with chronic kidney disease. Nephrol Dial Transplant 2011; 26: 2231–2237
27. Sherwin RS, Bastl C, Finkelstein FO *et al*. Influence of uraemia and hemodialysis on the turnover and metabolic effects of glucagon. J Clin Invest 1976; 57: 722–731
28. Hansen KB, Vilsboll T, Bagger JI *et al*. Increased postprandial GIP and glucagon responses, but unaltered GLP-1 response after intervention with steroid hormone, relative physical inactivity, and high-calorie diet in healthy subjects. J Clin Endocrinol Metab 2011; 96: 447–453
29. Rocha DM, Faloona GR, Unger RH. Glucagon-stimulating activity of 20 amino acids in dogs. J Clin Invest 1972; 51: 2346–2351
30. Calbet JA, MacLean DA. Plasma glucagon and insulin responses depend on the rate of appearance of amino acids after ingestion of different protein solutions in humans. J Nutr 2002; 132: 2174–2182
31. Lund A, Vilsboll T, Bagger JI *et al*. The separate and combined impact of the intestinal hormones, GIP, GLP-1, and GLP-2, on glucagon secretion in type 2 diabetes. Am J Physiol Endocrinol Metab 2011; 300: E1038–E1046
32. Heading RC, Nimmo J, Prescott LF *et al*. The dependence of paracetamol absorption on the rate of gastric emptying. Br J Pharmacol 1973; 47: 415–421
33. Strid H, Simren M, Stotzer PO *et al*. Delay in gastric emptying in patients with chronic renal failure. Scand J Gastroenterol 2004; 39: 516–520
34. Wright RA, Clemente R, Wathen R. Gastric emptying in patients with chronic renal failure receiving hemodialysis. Arch Intern Med 1984; 144: 495–496
35. Wettergren A, Schjoldager B, Mortensen PE *et al*. Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. Dig Dis Sci 1993; 38: 665–673
36. Miholic J, Orskov C, Holst JJ *et al*. Emptying of the gastric substitute, glucagon-like peptide-1 (GLP-1), and reactive hypoglycemia after total gastrectomy. Dig Dis Sci 1991; 36: 1361–1370
37. Vilsboll T, Toft-Nielsen MB, Krarup T *et al*. Evaluation of beta-cell secretory capacity using glucagon-like peptide 1. Diabetes Care 2000; 23: 807–812
38. Porte D, Jr. Banting lecture 1990. Beta-cells in type II diabetes mellitus. Diabetes 1991; 40: 166–180

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