GLP-1 Restores Altered Insulin and Glucagon Secretion in Posttransplantation Diabetes

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OBJECTIVE

Development of posttransplantation diabetes (PTDM) is characterized by reduced insulin secretion and sensitivity. We aimed to investigate whether hyperglucagonemia could play a role in PTDM and to examine the insulinotropic and glucagonostatic effects of the incretin hormone glucagon-like peptide 1 (GLP-1) during fasting and hyperglycemic conditions, respectively.

RESEARCH DESIGN AND METHODS

Renal transplant recipients with (n = 12) and without (n = 12) PTDM underwent two separate experimental days with 3-h intravenous infusions of GLP-1 (0.8 pmol/kg/min) and saline, respectively. After 1 h of infusion, a 2-h hyperglycemic clamp (fasting plasma glucose + 5 mmol/L) was established. Five grams of arginine was given as an intravenous bolus 10 min before termination of the clamp.

RESULTS

Fasting concentrations of glucagon (P = 0.92) and insulin (P = 0.23) were similar between the groups. In PTDM patients, glucose-induced glucagon suppression was significantly less pronounced (maximal suppression from baseline: 43 ± 12 vs. 65 ± 12%, $P < 0.001$), while first- and second-phase insulin secretion were significantly lower. The PTDM group also exhibited a significantly lower insulin response to arginine (P = 0.01) but similar glucagon and proinsulin responses compared with control subjects. In the preclamp phase, GLP-1 lowered fasting plasma glucose to the same extent in both groups but reduced glucagon only in PTDM patients. During hyperglycemic clamp, GLP-1 reduced glucagon concentrations and increased first- and second-phase insulin secretion in both groups.

CONCLUSIONS

PTDM is characterized by reduced glucose-induced insulin secretion and attenuated glucagon suppression during a hyperglycemic clamp. Similar to the case in type 2 diabetes, GLP-1 infusion seems to improve (insulin) or even normalize (glucagon) these pathophysiological defects.

In renal transplant recipients, cardiovascular disease persists as the leading cause of premature death (1). Development of posttransplantation diabetes (PTDM) is associated with further increased cardiovascular risk and mortality (2–4). PTDM is primarily believed to be a variant of type 2 diabetes possibly induced by immunosuppressive therapy (5) and/or viral infections (e.g., cytomegalovirus and hepatitis C) that reduce both insulin secretion and insulin sensitivity (6). Importantly, the risk of PTDM can be significantly reduced by proper dosing of the immunosuppressive

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agents (7). In nontransplanted patients, type 2 diabetes is characterized by insulin resistance and β-cell failure in addition to inappropriate α-cell function that result in fasting and postprandial hyperglucagonemia (8), both of which contribute to the hyperglycemic state of the patients (9). Hyperglucagonemia was recently demonstrated in uremic patients with impaired glucose tolerance (10). However, some aspects of the pathophysiology underlying the impaired glucose metabolism in renal transplant recipients with PTDM are still unclear.

The incretin hormone glucagon-like peptide 1 (GLP-1) is an insulinotropic peptide hormone secreted from enteroendocrine mucosal cells in response to food intake (11). GLP-1 also exerts glucagonostatic properties and contributes to suppress plasma concentrations of glucagon during oral glucose administration (12). Hyperglycemic clamp investigations with concomitant infusions of GLP-1 and placebo (saline), respectively, allow a thorough characterization of both α-cell and β-cell function. We aimed to investigate whether hyperglucagonemia could play a role in PTDM and to examine the insulinotropic and glucagonostatic effects of GLP-1 during fasting and hyperglycemic conditions, respectively.

Table 1—Patient characteristics

	PTDM, *n* = 11	Control subjects, *n* = 12	*P*
Age (years)	63 (39–70)	66 (47–77)	0.39
Male/female sex	9/2	10/2	
BMI (kg/m^2)	26.6 (25.8–29.6)	25.6 (24.2–30.5)	0.17
Renal transplantation			
Years after transplantation	2.5 (3.3)	1.5 (0.7)	0.02
Preemptive transplantation (yes/no)	4/7	4/8	
Donor (LD/DD)	4/7	4/8	
Prednisolone (mg/day)	4.8 ± 0.8	4.6 ± 1.0	0.67
Clinical measures			
Systolic blood pressure (mmHg)	145 ± 10	139 ± 15	0.12
Diastolic blood pressure (mmHg)	81 ± 9	79 ± 10	0.45
Laboratory results			
HbA_{1c} (%)	7.0 ± 0.6	5.8 ± 0.3	<0.001
HbA_{1c} (mmol/mol)	53 ± 6.6	40 ± 3.3	<0.001
HOMA-IR	6.52 ± 3.45	3.38 ± 1.88	0.007
eGFR (mL/min/1.73 m^2)	69 ± 12	62 ± 16	0.09
Total cholesterol (mmol/L)	4.6 ± 1.0	4.9 ± 0.9	0.29
HDL cholesterol (mmol/L)	1.3 ± 0.5	1.5 ± 0.3	0.02
LDL cholesterol (mmol/L)	2.6 ± 0.7	2.9 ± 0.8	0.11
Triglycerides (mmol/L)	2.6 ± 1.6	1.5 ± 0.8	0.007

Age is presented as median (range), and BMI and years after transplantation are presented as median (interquartile range). The rest of the data are presented as proportions or mean ± SD, calculated as the mean of the two examination days. DD, deceased donor; LD, living donor.

RESEARCH DESIGN AND METHODS

Patients

We performed a single-center study and included 24 renal transplant recipients (12 with PTDM and 12 without diabetes). All patients were Caucasians and matched for age, sex, BMI and renal function (characteristics presented in Table 1). Potential participants with PTDM were identified by routine screening in the outpatient clinic (fasting plasma glucose [FPG] ≥7.0 mmol/L and/or a 2-h postchallenge plasma glucose ≥11.1 mmol/L during a 75-g oral glucose tolerance test). Inclusion criteria were as follows: adult renal transplant recipient, >1 year posttransplant with stable renal function (<20% deviation in serum creatinine within last 2 months), stable prednisolone dose (maximum 5 mg/day) over the last 3 months, and BMI in the range of 18.5 to 29.9 kg/m^2. Exclusion criteria were severe liver disease, pancreatitis (chronic or acute), previous bowel resection, inflammatory bowel disease, malignancy (previous or actual), estimated glomerular filtration rate (eGFR) <25 mL/min/1.73 m^2, pregnancy, and breast-feeding. The patients were recruited from October 2014 to February 2015.

Study Design

The included patients underwent two experimental days separated by 2–4 weeks. On each experimental day, the participants met in the fasting state (10-h fast including liquids and tobacco). After fasting blood sampling, the patients were randomized to continuous unblinded intravenous infusion of GLP-1 (0.8 pmol/kg/min) or 0.9% saline (placebo), which was initiated at time 0 min. At time 60 min, a 2-h hyperglycemic clamp was initiated, where plasma glucose was elevated by 5 mmol/L from each individual FPG in both groups. This was done to mimic glucose variations in the PTDM group during daytime. At time 170 min, 5 g i.v. arginine was injected over a 1-min period as shown in Fig. 1. All patients were instructed to maintain usual exercise and diet habits during the study period. Any antidiabetes agents were washed out for 7 days before each experimental day. The study was performed according to the Declaration of Helsinki and was approved by the Regional committee for Medical Research Ethics, Norway, and evaluated by the Health Region South and The Data Inspectorate prior to the study start.

Study Procedures

Blood Samples

Patients were investigated in the recumbent position. A catheter was placed in an antecubital vein wrapped in a heating pad for sampling of arterialized blood (13). Fasting blood samples for determination of glucose, glucagon, proinsulin, and insulin were drawn before initiation of intravenous infusion of GLP-1/isotonic saline. Blood samples for measurement of glucagon and insulin were drawn at 0, 10, 20, 30, 45, 60, 70, 80, 90, 105, 120, 150, and 180 min (Fig. 1). Blood was sampled into prechilled 9 mL EDTA vacutainers for analysis of glucagon. A specific dipeptidyl peptidase-4 inhibitor (valine pyrrolidide, final concentration 0.01 mmol/L) was added to the EDTA vacutainers before blood was drawn. Blood for analysis of proinsulin and insulin was sampled in 2.5 mL serum separation tubes vacutainers. The EDTA vacutainers were kept on ice before and after blood sampling and centrifuged for 20 min at 1,200*g* and 4°C, and plasma was distributed into cryotubes and stored at −20°C until analysis. Blood in the serum separation tubes vacutainers was

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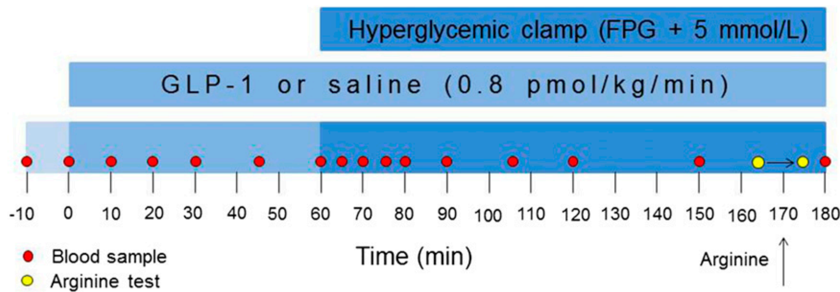

Figure 1—Study design. The vertical arrow indicates arginine infusion over 1 min at 170 min.

left to coagulate at room temperature before centrifuging for 10 min at 1,800*g*. Serum was distributed into cryotubes and stored at −20°C until analysis. During the hyperglycemic clamp, plasma glucose was measured bedside every 5 min in fresh whole blood.

GLP-1 Administration

Lyophilized GLP-1 (7-36)amide (100 μg) was reconstituted in 1.0 mL 0.9% saline at room temperature immediately before start of the experiment. The GLP-1 infusion consisted of 42.5 nmol/mL GLP-1 (7-36)amide, 12.5 mL 5% human albumin and isotonic saline was added to a total volume of 50 mL. On each study day, a catheter was also inserted in the contralateral antecubital vein and the continuous GLP-1/saline infusion was started at time 0 min and terminated at time 180 min.

Hyperglycemic Clamp

The hyperglycemic clamp was started at time 60 min, where a body weight–adjusted (200 mg/kg i.v.) bolus of 20% glucose was given over 5 min to quickly increase plasma glucose to FPG +5 mmol/L. Plasma glucose was kept at this level by adjustment of the infusion rate of a 20% glucose solution according to bedside plasma glucose measured every 5 min (14).

Arginine Stimulation Test

At time 170 min, i.e., during hyperglycemia, 5 g i.v. arginine was injected over 1 min (15). Prestimulus blood samples were taken at times 165 and 169 min, and additional blood samples were collected at times 172, 173, 174, and 175 min. After the clamp investigations, the patients received a meal to avoid hypoglycemia.

Analyses

Bedside blood glucose concentrations were measured in fresh blood samples with a portable plasma-calibrated glucose analyzer (Glucose 201 RT System, Hemocue, Ängelholm, Sweden, which fulfills the in vitro diagnostic medical devices directive 98/79/EC). For glucagon analysis (Millipore, Billerica, MA), plasma samples were assayed using antibody code no. 4305, raised in the laboratory of J.J.H., directed against the C terminal of the glucagon molecule as previously described (16). The sensitivity of the glucagon assay is 3 pmol/L and intra-assay coefficient of variation is 8% (16). ELISA kits based on the sandwich principle were used for quantitative measurement of intact serum proinsulin (EIA-1560) and insulin (EIA-2935) concentrations (DRG International, Springfield, NJ). The proinsulin assay had no cross-reactivity with insulin or vice versa.

Calculations

Results are expressed as mean ± SD unless otherwise stated. Fasting levels of plasma glucose, glucagon, and insulin were assessed as the mean of 0-min samples before infusion of GLP-1/saline from both experimental days. Area under the concentration versus time curve (AUC) was calculated by the trapezoidal rule. AUCs were evaluated in the basal period from 0 to 60 min ($AUC_{0–60}$), also referred to as baseline, and in the hyperglycemic period, but before the arginine stimulation test, from 60 to 169 min ($AUC_{60–169}$). Nadir glucagon and peak insulin values were used to describe maximal suppression of glucagon and maximal stimulation of insulin, respectively, as relative to baseline. The acute glucagon, proinsulin, and insulin secretory response to arginine was calculated as the mean of the plasma glucagon (acute glucagon response [AGR]), proinsulin (acute proinsulin response [APR]), and insulin (acute insulin response [AIR]) concentrations, respectively, at 2–5 min after the arginine injection minus the mean of the prestimulus concentrations (17). First-phase (65–80 min) and second-phase (150–169 min) insulin secretion during the hyperglycemic clamp period were evaluated as $AUC_{insulin}$/min in the respective periods. Insulin sensitivity index (ISI [*M*/*I*]) (18) was evaluated on the placebo day and calculated by dividing the mean glucose infusion rate (*M* [in μmol/kg/min]) in the stable phase at time 150–169 min during hyperglycemic clamp by the mean insulin concentration (*I* [in pmol/L]) in the same interval. Insulin resistance was also evaluated by HOMA (HOMA-IR) and calculated as HOMA-IR = (fasting insulin [μIU/mL] × FPG [mmol/L])/22.5. eGFR was calculated by the MDRD formula (19). Since estimation of the proinsulin-to-insulin ratio within the secretory granules of the β-cell is most reliable after acute stimulation of secretion, the proinsulin secretory ratio (PISR) was examined in acute response to arginine and calculated as APR/AIR × 100 (20).

Statistical Considerations

Number of Patients

According to the type 2 diabetes literature, we assumed that the PTDM group would have 30 ± 15% higher baseline plasma glucagon concentrations than the control group, with a corresponding difference in GLP-1–induced suppression of glucagon (21). Twenty patients were needed to assure a power of 90% to show this difference at a 5% significance level. We therefore included 24 patients (12 patients in each group) to allow for a 20% dropout rate.

Analysis Plan

Comparisons within and between groups, respectively, were performed by paired and unpaired sample *t* tests as appropriate and presented as means ± SD with *P* values. For data that were not normally distributed, the statistical analyses were performed on logarithmic-transformed data. Data that remained skewed after logarithmic transformation were analyzed by Mann-Whitney *U* test and presented as median (interquartile or absolute range). Correlations were analyzed by Pearson correlation. All statistical analyses were performed using SPSS for Windows (version 22.0; SPSS, Chicago, IL).

RESULTS

Demographic and Clinical Data

All included patients completed the study. Data from one patient in the

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PTDM group were excluded from the statistical analyses owing to normalization of glucose values since last visit in the outpatient clinic. Patient demographics and clinical and laboratory data are shown in Table 1. PTDM patients (*n* = 11) were comparable with control subjects (*n* = 12) with regard to all demographic variables except for time after transplantation, which was significantly longer in the PTDM group. At the time of inclusion, mean duration of PTDM was 4.3 ± 4.5 years and seven of the patients in the PTDM group had received long-term treatment with oral antidiabetes agents (sitagliptin [*n* = 3], glimepiride + sitagliptin [*n* = 1], glipizide [*n* = 2], and metformin [*n* = 1]). None received insulin treatment. The immunosuppressive treatments were comparable in the two groups, and all included patients except one in each group received a regimen that consisted of prednisolone, mycophenolate mofetil, and a calcineurin inhibitor (tacrolimus) (*n* = 15 [9 in the control group and 6 in the PTDM group]), cyclosporine (*n* = 6 [2 in the control group and 4 in the PTDM group]). Both HOMA-IR and HbA_{1c} values were significantly higher in the PTDM group.

Glucose

Based on the World Health Organization diagnostic criteria for impaired fasting glucose (IFG) (FPG between 6.1 and 6.9 mmol/L), none of our patients had IFG. However, with application of the American Diabetes Association diagnostic criteria of IFG (FPG between 5.6 and 6.9 mmol/L), two of the patients in the control group would have been categorized with IFG (FPG of 5.7 and 6.0 mmol/L, respectively). FPG was significantly higher in the PTDM group, which resulted in significantly higher AUCs in both the basal and hyperglycemic periods (Table 2). Infusion of GLP-1 reduced AUCs in both periods. Plasma glucose in the basal period was lowered by GLP-1 ($P \leq 0.001$) to the same extent in the PTDM group (−0.5 ± 0.7 mmol/L) as in control subjects (−0.7 ± 0.3) ($P = 0.83$).

Glucagon

There were no significant differences between the groups in fasting plasma concentrations of glucagon (PTDM 8.6 ± 2.4 pmol/L and control subjects 9.2 ± 3.8 pmol/L, $P = 0.92$). The PTDM group had significantly lower glucose-induced glucagon suppression in the hyperglycemic period (during clamp conditions) than control subjects. Maximal suppression from baseline was 43 ± 12% in the PTDM group vs. 65 ± 12% in control subjects ($P < 0.001$). There was no difference in AGR to arginine between the groups.

Table 2—Glucose, glucagon, and insulin

	PTDM, *n* = 11	Control subjects, *n* = 12	*P*
Plasma glucose			
Fasting glucose (mmol/L)	7.2 ± 1.0	5.0 ± 0.7	<0.001
$AUC_{0–60}$, placebo (mmol · min/L)	435 ± 58	318 ± 25	<0.001
$AUC_{0–60}$, GLP-1 (mmol · min/L)	403 ± 66‡	277 ± 28‡	<0.001
$AUC_{0–60\ /60}$, GLP-1 minus saline (mmol/L)	−0.5 ± 0.7	−0.7 ± 0.3	0.83
$AUC_{60–169}$, placebo (mmol · min/L)	1,356 ± 133	1,092 ± 82	<0.001
$AUC_{60–169}$, GLP-1 (mmol · min/L)	1,291 ± 123†	1,035 ± 92†	<0.001
Plasma glucagon			
Fasting glucagon (pmol/L)	8.6 ± 2.4	9.2 ± 3.8	0.92
$AUC_{0–60}$, placebo (pmol · min/L)	476 ± 156	509 ± 266	1.00
$AUC_{0–60}$, GLP-1 (pmol · min/L)	387 ± 162‡	446 ± 146	0.18
$AUC_{60–169}$, placebo (pmol · min/L)	572 ± 196	441 ± 245	0.09
$AUC_{60–169}$, GLP-1 (pmol · min/L)	376 ± 202‡	334 ± 129†÷	0.66
Maximal suppression placebo (% from baseline)	43 ± 12	65 ± 12	<0.001
Maximal suppression GLP-1 (% from baseline)	55 (28)‡÷	72 (14)	0.04
AGR, placebo (pmol/L)	11.5 ± 6.8	13.6 ± 5.6	0.35
AGR, GLP-1 (pmol/L)	10.5 ± 6.4	10.8 ± 5.0†	0.76
Serum insulin			
Fasting insulin (pmol/L)	159 ± 82	124 ± 52	0.23
$AUC_{0–60}$, placebo (pmol · min/L)	8,316 ± 4,153	6,164 ± 2,883	0.15
$AUC_{0–60}$, GLP-1 (pmol · min/L)	17,189 ± 10,402‡	10,947 ± 5,296†	0.10
$AUC_{60–169}$, placebo (pmol · min/L)	22,001 ± 9,541	41,531 ± 28,457	0.03
$AUC_{60–169}$, GLP-1 (pmol · min/L)	150,666 ± 125,480‡	235,577 ± 156,144‡	0.06
Maximal stimulation placebo (% from baseline)	79 ± 55	363 ± 214	<0.001
Maximal stimulation GLP-1 (% from baseline)	633 ± 436‡	1,967 ± 1,188†	0.001
AIR, placebo (pmol/L)	697 ± 299	1,267 ± 630	0.01
AIR, GLP-1 (pmol/L)	889 ± 543÷	1,420 ± 524	0.03
Secr1.phase, placebo (pmol/L)	145 ± 53	403 ± 326	0.001
Secr1.phase, GLP-1 (pmol/L)	559 ± 358‡	1,167 ± 1,122‡	0.02
Secr2.phase, placebo (pmol/L)	244 ± 130	464 ± 327	0.03
Secr2.phase, GLP-1 (pmol/L)	2,270 ± 2,155‡	3,159 ± 1,983‡	0.09

Data are mean ± SD or median (interquartile range). Fasting values are evaluated as mean of 0 min samples from both experimental days. AGR and AIR were calculated as the mean of the plasma glucagon and serum insulin concentrations at 2–5 min after the arginine injection minus the mean of the prestimulus (−5 min and −1 min) concentrations. Secr1.phase, first-phase insulin secretion (65–80 min), and Secr2.phase, second-phase insulin secretion (150–169 min), evaluated as $AUC_{insulin}$/min in the respective time periods. ÷One missing variable is denoted. Significance of analysis by paired *t* test within the groups reported as: ‡$P < 0.05$, †$P \leq 0.001$.

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Concomitant GLP-1 infusion in the basal period resulted in a significant reduction in glucagon levels in the PTDM group (−22 ± 15% reduction in $AUC_{0–60}$, P = 0.007) but not in the control group. In the hyperglycemic period, GLP-1 resulted in a significant suppression of glucagon in both groups. GLP-1 reduced AGR significantly by 3.1 ± 2.7 pmol/L ($P < 0.05$) in the control group but not in the PTDM group (Table 2 and Fig. 2).

Insulin

Fasting serum insulin concentrations did not differ between the groups (PTDM 159 ± 82 pmol/L and control subjects 124 ± 52 pmol/L, P = 0.23). The PTDM group had a significantly lower capacity to stimulate insulin secretion (maximal stimulation from baseline 79 ± 55 vs. 363 ± 214%, $P < 0.001$) in addition to a lower first-phase (P = 0.001) and second-phase (P = 0.03) insulin secretion, as shown in Table 2 and Fig. 2. AIR to arginine was also significantly lower in the PTDM group than in control subjects (697 ± 299 vs. 1,267 ± 630 pmol/L, respectively, P = 0.01).

The insulin secretion in the basal period was significantly increased by GLP-1 in both groups ($AUC_{0–60}$ incretion 102 ± 62%, P = 0.003, in PTDM and 78 ± 48%, $P < 0.001$, in control subjects). In the hyperglycemic period, GLP-1 resulted in a significant increase in first- and second-phase insulin secretion in both groups. First-phase insulin secretion remained significantly lower in the PTDM group (559 ± 358 vs. 1,167 ± 1,122 pmol/L, P = 0.02). Maximal insulin stimulation from baseline also remained significantly lower in the PTDM group: relative increment of 555 ± 407 vs. 1,604 ± 1,096% (P = 0.004). Infusion of GLP-1

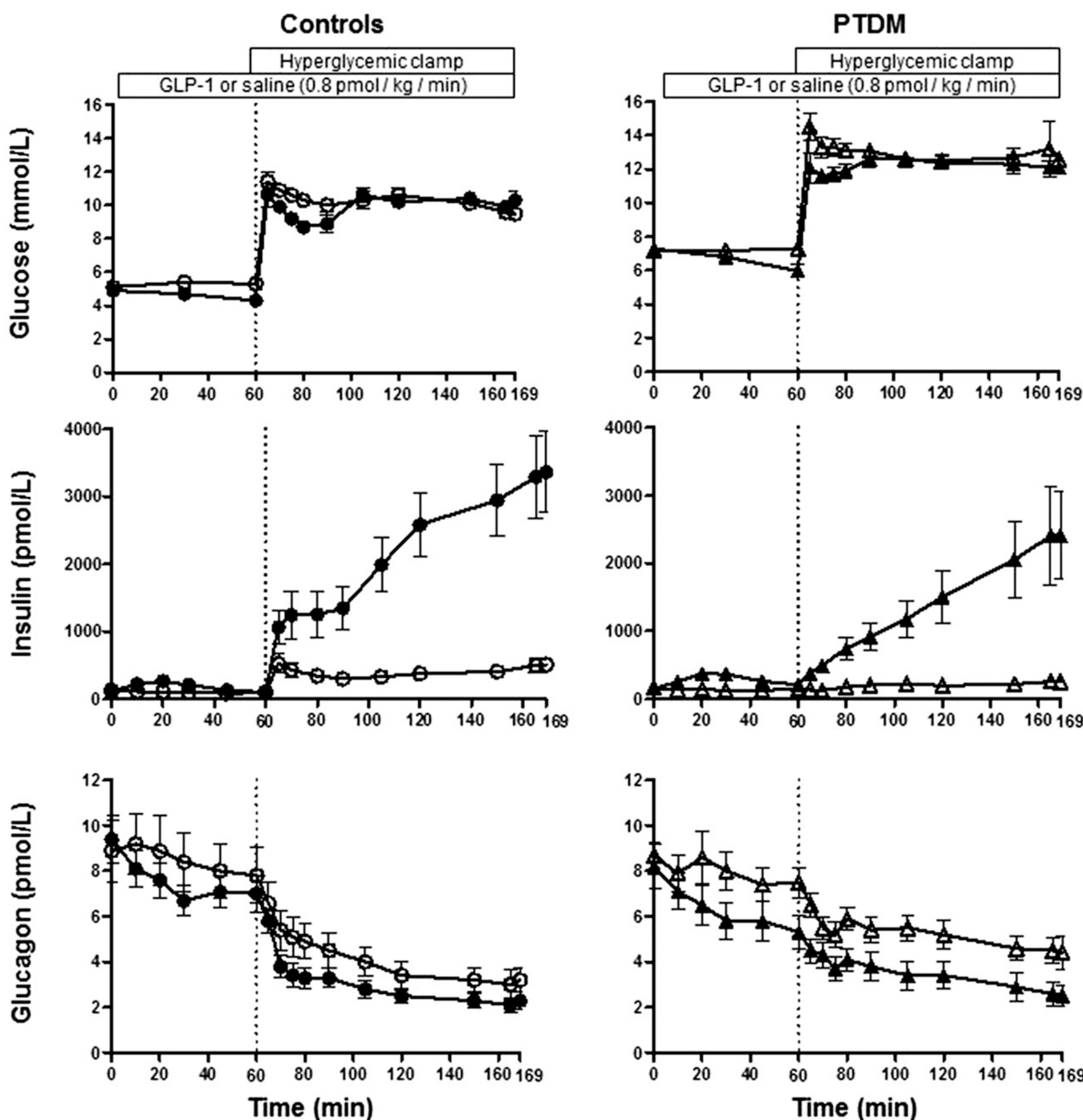

Figure 2—Glucose, insulin, and glucagon. Glucose, insulin, and glucagon concentrations in the control group (circles) and the PTDM group (triangles) with GLP-1 (closed symbols) or saline (open symbols). The data are presented as mean ± SEM.

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did not significantly increase AIR, but it remained significantly lower in the PTDM group than in the control group (P = 0.03).

Correlation

Glucagon and insulin secretion were significantly correlated (30–169 min) within respective groups: $r = -0.809$ in the PTDM group (P = 0.001) and $r = -0.903$ in the control group ($P < 0.001$). The secretions were highly correlated during concomitant GLP-1 infusion (PTDM $r = -0.915$, $P < 0.001$; control subjects $r = -0.917$, $P < 0.001$).

Proinsulin

Fasting proinsulin tended to be higher in the PTDM group (7.9 ± 11.9 pmol/L) compared with that in control subjects (4.1 ± 4.4 pmol/L, P = 0.18). Fasting proinsulin-to-insulin ratio was, however, not significantly different between the groups: 4.7 ± 4.4 vs. 3.0 ± 2.3 pmol/L (P = 0.35). The APR tended to be lower in the PTDM group than in control subjects (3.3 ± 4.0 vs. 15.3 ± 18.7 pmol/L, P = 0.06). GLP-1 increased APR significantly within both groups (to 18.4 ± 22.1 pmol/L, $P \leq 0.001$, in the PTDM group and to 31.4 ± 54.7 pmol/L, $P < 0.05$, in control subjects). There were no differences in PISR between the groups, and GLP-1 did not increase PISR significantly (data not shown).

Insulin Sensitivity

Insulin sensitivity (ISI) was calculated in the time period 150–169 min during the hyperglycemic clamp. There was no significant difference in median ISI between the PTDM group (0.070 μmol/kg/min per pmol/L [interquartile range 0.113]) and control group (0.069 μmol/kg/min per pmol/L [0.115], P = 0.67). However, HOMA-IR was significantly higher in the PTDM group (P = 0.007) (Table 1).

CONCLUSIONS

We show that renal transplant recipients with PTDM, concurrent with reduced insulin secretion, have a reduced ability to suppress circulating glucagon levels during a hyperglycemic clamp. This imbalance in the insulin-glucagon axis during hyperglycemia resembles that seen in patients with type 2 diabetes (21,22), and we suspect that this bihormonal defect increases hepatic glucose production and, thus, plays an important role in PTDM pathophysiology. Importantly, our results also suggest that GLP-1 may improve this pathophysiological defect in PTDM.

There was no difference in fasting plasma concentration of glucagon between patients with PTDM and renal transplant recipients without diabetes, although FPG was slightly higher in the PTDM group. To the best of our knowledge, this is the first study to assess glucagon concentrations in patients with PTDM, and our findings are in apparent contrast to findings in nontransplanted patients with type 2 diabetes where fasting hyperglucagonemia and higher FPG have been reported (21–23). Since our patients only had mild hyperglycemia in the fasting state, one may speculate that the findings could have been more pronounced in a more advanced state of PTDM.

Elevation of proinsulin in serum is a reflection of impaired insulin biosynthesis in the β-cell, and elevated fasting proinsulin concentrations constitute a significant risk factor for development of PTDM (24). In the current study, neither fasting proinsulin-to-insulin ratio nor PISR in response to arginine was significantly different between the groups, indicating appropriate biosynthesis and secretion of proinsulin. These data support that the reduced β-cell secretory capacity is best explained by decreased functional β-cell mass rather than impaired biosynthesis of insulin (15).

It has previously been found that patients with PTDM in general are characterized with a more or less normal FPG with an isolated postprandial hyperglycemia (25). This is in contrast to patients with type 2 diabetes, who tend to have a better correlation between FPG and postprandial hyperglycemia (26). In the current study, we did not find elevated fasting glucagon concentrations, but reduced glucose-induced glucagon suppression during hyperglycemic clamp. This could indicate that glucagon plays a role in the postprandial hyperglycemia frequently seen in PTDM. Patients with end-stage renal disease have fasting glucagon concentrations about three times higher than healthy individuals (10,27). The results in the current study are consistent with the finding that fasting hyperglucagonemia in uremia is reversed by renal transplantation (28). It is demonstrated that the hyperglucagonemia seen in renal disease is caused by accumulated amounts of circulating N-terminally elongated forms of glucagon, including proglucagon (1-61), but the mechanism behind this is not known (16). Overstimulation of the α-cells by glucose-dependent insulinotropic polypeptide (GIP) may be an explanation (29).

The incretin hormones GLP-1 and GIP are responsible for up to 70% of the insulin response after ingestion of glucose (the incretin effect) in healthy individuals (30). Patients with type 2 diabetes have impairments in the incretin system, and furthermore, they exhibit elevated plasma glucagon levels that are nonsuppressible the first hour after oral glucose administration (23,31). The attenuated and delayed glucagon suppression has only been found after oral ingestion of glucose, while isoglycemic intravenous administration of glucose has resulted in more or less normal suppression of glucagon (22). In the current study, intravenous administration of glucose resulted in significantly lower glucagon suppression in the PTDM group than in control subjects (maximal suppression from baseline 43 ± 12 vs. 65 ± 12%, $P < 0.001$). This could be related to the lower first- and second-phase insulin secretion in the PTDM group. The secretion of glucagon was found to be inversely correlated to the secretion of insulin. It has previously been reported that there must be an adequate stimulation of insulin secretion in order to get an adequate suppression of glucagon, since impaired insulin secretion leads to loss of intraislet insulin-driven suppression of glucagon secretion (32). Furthermore, a recent study found that the sodium-glucose cotransporter 2 (SGLT2) is expressed in glucagon-secreting α-cells and that sodium-glucose cotransport by SGLT2 is essential for appropriate regulation of glucagon secretion (33).

We clamped the patients in both groups at plasma glucose levels 5 mmol/L above their individual FPG. In this way, all patients had the same absolute increment in plasma glucose. We also infused GLP-1 in physiological doses to obtain plasma concentrations similar to those seen after a meal in healthy individuals (34). Concomitant GLP-1 infusion during the hyperglycemic clamp elicited markedly lower glucagon

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responses as well as higher insulin responses compared with saline, which reflect the potent glucagonostatic and insulinotropic effects of GLP-1. Although GLP-1 had significant insulinotropic effects in both groups, the effect on first-phase insulin secretion was lower in the PTDM group than in control subjects, with AUC increments of 415 ± 313 and 763 ± 834 pmol/L (P = 0.09), respectively. This was seen in addition to a significant lower maximal stimulation from baseline (555 ± 407% in the PTDM group and 1,604 ± 1,096% in control subjects [P = 0.004]). In contrast, GLP-1 exerted similar glucagonostatic effects in the PTDM group and control subjects during hyperglycemic clamp. This observation is in accordance with findings in patients with type 2 diabetes (35). However, in the basal period GLP-1 reduced plasma glucagon only in the PTDM group. This is most likely due to the glucose-dependent glucagon-suppressive effect of GLP-1.

Development of PTDM contributes to increased cardiovascular disease and premature mortality in renal transplant recipients (2–4). It is therefore important to explore the pathophysiology of PTDM and expose targets of treatment to reduce hyperglycemia in a safe way. The number of oral drugs available for treatment of hyperglycemia in renal transplant recipients is limited because many recipients often have reduced renal function and because of the potential interactions with immunosuppressive drugs and adverse effects such as hypoglycemic events, which may increase the cardiovascular risk. Efficacy and safety of the dipeptidyl peptidase-4 inhibitors sitagliptin (36,37) and vildagliptin (38,39) have previously been documented in PTDM patients. The insulinotropic and glucagonostatic effects of GLP-1 described in the current study imply that GLP-1 analogues also could be an alternative in the treatment of PTDM. Short-term safety of GLP-1 treatment has recently been demonstrated in patients with type 2 diabetes treated with hemodialysis (40). In these patients, liraglutide plasma concentrations increased, so reduced treatment doses may be advisable in treatment of patients with PTDM and reduced glomerular filtration rate.

All included patients in the current study were Caucasians, so our data may not be representative for other patient populations. The study was not blinded and had a limited sample size. We included a control group of renal transplant recipients without diabetes, since they have been exposed to procedures and medication similar to those of the PTDM group. The changes in glucagon and insulin concentrations must be related to the prevailing plasma glucose concentrations in the two groups. Direct comparison of hormone concentrations between groups can therefore not be performed. However, it was evident from the saline infusions (placebo) that—relative to prevailing glucose concentrations—insulin secretion was disproportionally low and glucagon secretion was disproportionally high during the hyperglycemic state when PTDM patients were compared with renal transplant recipients without diabetes. Strength of the study was that the PTDM and control group were matched for age, sex, BMI, and renal function to minimize effect of confounders. Insulin sensitivity should ideally be measured by a hyperinsulinemic-euglycemic clamp (18). A surrogate estimate can be obtained during a hyperglycemic clamp by dividing the mean glucose infusion rate during the last hour of the hyperglycemic clamp by the mean plasma insulin concentration in the same interval. In the current study, the glucose infusion rate did not stabilize until 90 min into the hyperglycemic clamp. Therefore, the ISI was calculated for the last 19 min before the arginine stimulation test. We did not find a difference in the calculated ISI between the groups, and this could be due to insufficient stabilization of the insulin concentrations. Another surrogate estimate of insulin resistance, HOMA-IR, showed reduced insulin sensitivity in the PTDM group.

In conclusion, our findings suggest that the pathophysiology of PTDM, in addition to inadequate insulin secretion, involves impaired glucose-induced glucagon suppression in the hyperglycemic state and that exogenously delivered GLP-1 improves both deficiencies in renal transplant recipients with PTDM.

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