ORIGINAL ARTICLE

The impact of kidney transplantation on insulin sensitivity

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SUMMARY

To investigate the impact of kidney transplantation (KTx) on insulin sensitivity affecting glucose metabolism. 9 nondiabetic patients awaiting living donor KTx were examined prior to transplantation with an oral glucose tolerance test and a 3-h hyperinsulinaemic–euglycaemic clamp. The clamp was repeated 6 months after KTx. Nine age-, gender- and body mass index (BMI)-matched individuals with normal kidney function served as controls. Endogenous glucose production and glucose disappearance rate ($N = 6$) were measured in a subgroup of patients with corresponding controls. Results presented as mean [range]. Two patients had pretransplant prediabetes, whereas all others had normal glucose tolerance. After KTx, average glucose infusion rate to maintain euglycaemia during clamp declined significantly from 15.1 [9.1–23.7] to 9.8 [2.8–14.6] μmol/kg/min ($P < 0.01$) with 20.2 [9.9–33.7] μmol/kg/min in controls. Endogenous glucose production increased from 7.0 [4.8–8.5] to 9.4 [7.4–11.8] μmol/kg/min ($P < 0.05$) with 7.0 [−3.8 to 10.1] μmol/kg/min in controls. Glucose disappearance rate was unchanged (18.1 [12.9–24.5] vs. 17.1 [12.2–22.7] μmol/kg/min, NS) with 22.3 [14.6–34.3] in controls. In conclusion, insulin sensitivity is reduced 6 months after KTx and characterized mainly by impaired suppression of the endogenous glucose production.

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Key words
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Introduction

Severe uraemia has been established as a cause of insulin resistance which is clinically evident by the high incidence of glucose intolerance in patients awaiting kidney transplantation [1]. The insulin resistance has been characterized using clamping techniques with isotope tracers and shown to affect both peripheral glucose uptake and endogenous glucose production although some studies have failed to show the latter [2–4]. Interestingly, alleviation of uraemia by kidney transplantation seems to increase rather than decrease the risk of developing diabetes [5].

The glucometabolic disturbances leading to this post-transplantation diabetes mellitus (PTDM) have been less well characterized. Some studies have indicated that the primary cause is a beta-cell dysfunction rather than insulin resistance [6–10], while others suggest an aggravation of insulin resistance as the main cause [11,12]. As a possible shortcoming, these studies have either been cross-sectional [6,7] or have used indices of insulin

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sensitivity and beta-cell function [8–12] which are possibly affected by the reduced insulin clearance observed in renal impairment [13]. Furthermore, several indices are calculated using solely fasting values which seems inappropriate as prediabetic and diabetic plasma glucose levels are mostly observed postprandial in both uraemia [1] and PTDM [11,14].

The purpose of this study was to determine the change in insulin sensitivity following kidney transplantation using the gold standard method of hyperinsulinaemic–euglycaemic clamp technique. Furthermore, we sought to characterize this change using both glucose and glycerol tracers in order to measure insulin sensitivity on peripheral glucose uptake, endogenous glucose production and whole-body lipolysis. We hypothesized that the insulin sensitivity was impaired after kidney transplantation in nondiabetic patients.

Materials and methods

Subjects

Nine patients awaiting living donor kidney transplantation in Denmark at Department of Nephrology, Rigshospitalet ($N = 7$), Department of Nephrology, Odense University Hospital ($N = 1$) and Department of Nephrology, Herlev Hospital ($N = 1$) were included from October 2007 to March 2014. Exclusion criteria included diabetes (diagnosed according to WHO criteria [15]), previous transplantation, planned AB0-incompatible transplantation, treatment with peritoneal dialysis and daily intake of medication known to influence glucose metabolism (including pretransplant oral glucocorticoids and calcineurin inhibitors). Nine age-, gender- and BMI-matched subjects with normal kidney function were recruited by advertising and served as controls.

Study design and experimental procedures

All participants included were screened with an oral glucose tolerance test with plasma glucose samples drawn before and 2 h after ingestion of 75 g glucose dissolved in 250 ml water. A hyperinsulinaemic–euglycaemic glucose clamp was performed before (PreTx) and 6 months after transplantation (PostTx) and once in control subjects (Ctrl): after an 8-h overnight fast and refrain from morning medications, a cubital vein catheter was inserted for infusions and a venous catheter in the contralateral forearm/hand for blood samples, heated to approximately 50 °C by a heating blanket to arterialize the blood. After baseline samples were drawn, a bolus of the tracers [6,6-$^2H_2$]glucose (17.6 μmol/kg) and [1,1,2,3,3-$^5H_2$]glycerol (1.5 μmol/kg; Cambridge Isotope Laboratories Inc., Tewksbury, MA, USA) was administered followed by a constant tracer infusion (glucose: 0.4 μmol/kg/min and glycerol: 0.1 μmol/kg/min).

After 2 h (Time 120 min), an additional infusion of human insulin (20 mU/m^2/min, Actrapid; Novo Nordisk, Copenhagen, Denmark) was administered while increasing the rate of glucose tracer infusion to 0.6 μmol/kg/min and decreasing the rate of glycerol tracer to 0.05 μmol/kg/min. From time 120 to 300 min, blood samples were collected 5–15 min apart and immediately analysed for plasma glucose (ABL; Radiometer, Brønshøj, Denmark). A variable infusion of 200 g/l glucose was adjusted accordingly to target a plasma glucose of 5 mmol/l. Blood samples for later analyses were drawn at Time −5, 0, 90, 105, 120, 150, 180, 210, 240, 270 and 300 min. Results from glucose tracers were obtained in six patients with corresponding controls and results from glycerol tracers in five patients with corresponding controls. Glycerol concentrations were obtained in all participants. Body composition was determined before and 6 months after transplantation and once in control subjects by a Dual-energy X-ray absorptiometry (DXA) scan (XR-46; Norland, Fort Atkinson, WI, USA).

Analyses

Baseline blood samples were analysed immediately as routine samples at Department of Clinical Biochemistry, Rigshospitalet. Serum insulin was also analysed immediately with a sandwich electro-chemiluminescence immunoassay (ECLIA) method (5% maximum combined intra- and interassay variability; Elecsys, Roche Diagnostics GmbH, Mannheim, Germany). Glucose and glycerol enrichments were analysed as previously described [16]. Blood samples for later analyses were centrifuged after collection for 10 min at 2000 *g* and 4 °C. Serum samples were stored 30 min in room temperature prior to centrifugation. Plasma and serum samples were frozen to and stored at −20 °C for subsequent *en bloc* analyses. Plasma glucagon was analysed with a sandwich enzyme-linked immunosorbent assay (ELISA) method (9.5% maximum combined intra- and interassay variability; Mercodia AB, Uppsala, Sweden) which has been validated in uraemic patients [17].

Stable isotope tracer calculations

Endogenous glucose production during clamp (glucose rate of appearance at time t, Glucose $Ra_{Endo}(t)$) and

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peripheral glucose uptake during clamp (glucose rate of disappearance at time t, Glucose Rd(t)) were calculated using single pool, non-steady-state kinetics with an effective volume of glucose distribution of 70 ml/kg [18]:

$$\text{Glucose Ra}_{\text{Endo}}(t) = \frac{F - pV_{\text{d}} \cdot \bar{C} \cdot \frac{\Delta E}{\Delta t}}{\bar{E}} - \overline{\text{GIR}}$$

$$\text{Glucose Rd}(t) = \frac{F - pV_{\text{d}} \cdot \bar{C} \cdot \frac{\Delta E}{\Delta t}}{\bar{E}} - \frac{pV_{\text{d}} \cdot \Delta C}{\Delta t}$$

where F is the glucose tracer infusion rate (μmol/kg/min), pV_{d} the effective volume of glucose distribution (ml/kg), $\bar{C}$ the mean glucose concentration from sample collected at time t and the previous sample (μmol/l), ΔE the difference in glucose enrichment from sample collected at time t and the previous sample, Δt the time difference from the previous sample to time t (min), $\bar{E}$ the mean glucose enrichment from sample collected at time t and the previous sample, $\overline{\text{GIR}}$ the mean nonenriched glucose infusion rate from time t and the rate where the previous sample was drawn (μmol/kg/min) and ΔC is the difference in glucose concentration from sample collected at time t and the previous sample.

The glucose rate of appearance and rate of disappearance in the basal period were calculated using steady-state kinetics.

Whole-body lipolysis during clamp (glycerol rate of appearance at time t, Glycerol Ra(t)) was calculated using single pool, non-steady-state kinetics with an effective volume of glycerol distribution of 330 ml/kg [19]:

$$\text{Glycerol Ra}(t) = \frac{F - pV_{\text{d}} \cdot \bar{C} \cdot \frac{\Delta E}{\Delta t}}{\bar{E}} - F$$

where F is the glycerol tracer infusion rate (μmol/kg/min), pV_{d} the effective volume of glycerol distribution (ml/kg), $\bar{C}$ the mean glycerol concentration from sample collected at time t and the previous sample (μmol/l), ΔE the difference in glycerol enrichment from sample collected at time t and the previous sample, Δt the time difference from the previous sample to time t (min) and $\bar{E}$ the mean glycerol enrichment from sample collected at time t and the previous sample.

Glycerol rate of appearance in the basal period was calculated using steady-state kinetics.

Statistical analysis

Differences were analysed using Student's t-test – paired within patients (PostTx – PreTx) and unpaired between patients before transplantation and controls (PreTx – Ctrl). Distributions were graphically evaluated prior to test for approximate normality, and folded F-test was used to evaluate equality of variances. The Satterthwaite correction was used in the presence of unequal variances. Average plasma glucose concentration and average glucose infusion rate during clamp were calculated as the total area under the curve (AUC) divided by time (180 min). Insulin concentrations in the basal period and glucagon and glycerol measurements during both basal and clamp period showed a log-normal distribution and were subsequently logarithmic transformed prior to analysis and results back-transformed. As a consequence, these values are in geometric means and differences are in relative units. Otherwise, results are expressed as means with either range or 95% confidence limits (Cl) unless otherwise stated. All results were analysed using SAS® 9.4 (SAS Institute Inc., Cary, NC, USA). P values < 0.05 were considered significant.

Study protocol

The study protocol was an amendment to a previous reported trial [11] and approved by the Scientific-Ethical Committee of the Capital Region of Denmark (H-KF-279825) and by the Danish Data Protection Agency (2006-41-5640). Written informed consent was obtained from all participants before inclusion, and the study was conducted according to the latest revision of the Helsinki Declaration.

Results

Patient characteristics and fasting values

Kidney diseases in patients included glomerulonephritis ($N = 3$), focal segmental glomerulosclerosis ($N = 1$), polycystic kidney disease ($N = 1$), congenital urinary tract abnormality ($N = 1$), Alport syndrome ($N = 1$) and unknown ($N = 2$). Three patients had initiated chronic haemodialysis treatment 4, 9 and 14 months prior to transplantation. Patients and controls were similar according to age, gender and BMI (Table 1). Patients had a nonsignificant higher blood pressure than controls with eight patients receiving between one and three antihypertensive agents both prior to and after transplantation (excluding furosemide) (Table 1).

Immunosuppressive treatments

At the initiation of transplantation, the patients received immunosuppressive therapy including induction with basiliximab, cyclosporine ($N = 4$) or tacrolimus ($N = 5$),

Table 1. Baseline characteristics.

	PreTx	Ctrl
Demographics		
Age (years)	33 (19–63)	29 (23 – 45)
Gender (m/f)	7/2	7/2
Caucasian (*N*)	7	9
BMI (kg/m^2)	24.5 (21.7–28.1)	24.6 (21.7–27.6)
Clinical		
Systolic BP (mmHg)	138 (110–176)	123 (99–138)
Diastolic BP (mmHg)	83 (60–99)	76 (61–103)
Pulse (bpm)	69 (54–92)	66 (51–98)
AH treatment (*N*)	8	1
CVD (*N*)	2 (NYHA II)	0

PreTx, before transplantation; Ctrl, control subjects; BMI, body mass index; BP, blood pressure; bpm, beats per minute; AH, antihypertensive; CVD, cardiovascular disease; NYHA, New York Heart Association Functional Classification.

Values expressed as mean (range) or as number.

There were no significant differences ($P < 0.05$) between any baseline characteristics.

mycophenolate mofetil (MMF) or mycophenolic acid and prednisolone/methylprednisolone (one treated with glucocorticoid-free protocol). Following transplantation, prednisolone was tapered down individually to 5–12.5 mg/day (median: 7.5 mg) 6 months after transplantation. One patient had the immunosuppressive treatment changed from MMF to azathioprine due to abdominal pain, another changed from tacrolimus to everolimus due to a suspected impact on the graft function and a third changed from prednisolone to deflazacort. Mean blood trough levels of calcineurin/mTOR inhibitors 6 months after transplantation were 103 μg/l for cyclosporine [$N = 3$, excluding one patient on glucocorticoid-free protocol (B-cyclosporine, 2 h postdose: 767 μg/l)], 7.1 μg/l for tacrolimus ($N = 4$) and 6.4 μg/l for everolimus ($N = 1$). Four patients were suspected of having rejection in the period between clamp examinations, one due to a delayed graft function and three due to an increase in plasma creatinine. The patients received a short (1–3 days) treatment with methylprednisolone (250–500 mg/day) all within the first month after transplantation. Graft biopsies confirmed acute rejection grade 1b in one patient, borderline acute rejection in another but no histological signs of acute rejection in the remaining two patients.

Glucose concentrations and infusion rates

On the screening day prior to transplantation, one patient had impaired fasting glucose (plasma glucose 6.5 mmol/l) and another patient had impaired glucose tolerance (2-h plasma glucose 8.7 mmol/l), while the remaining participants had normal fasting glucose and normal glucose tolerance. Patients had significant higher 2-h plasma glucose value than controls [PreTx – Ctrl: 1.6 Cl (0.4–2.8) mmol/l] (Table 2). Glycated haemoglobin (HbA1c) increased slightly after transplantation (PostTx – PreTx: 0.2 Cl [0.04–0.4] % (2.2 Cl [0.4–4.0] mmol/mol)) and was similar in controls and patients before transplantation.

During the hyperinsulinaemic clamp, plasma glucose was regulated to similar concentration levels in patients before and after transplantation and in controls (Table 3). Insulin sensitivity measured by the average glucose infusion rate to maintain euglycaemia decreased significantly 6 months after transplantation [PostTx – PreTx: −5.3 Cl (−8.9 to −1.7) μmol/kg/min] and tended to be lower in patients before transplantation than in controls [PreTx – Ctrl: −5.1 Cl (−12.3 to 2.1) μmol/kg/min] (Table 3 and Fig. 1a).

Endogenous glucose production and peripheral glucose uptake

In the basal period, endogenous glucose production/peripheral glucose uptake measured by glucose rate of appearance/disappearance was similar in patients before and after transplantation and in controls [PostTx – PreTx: 0.5 Cl (−1.1 to 2.2) μmol/kg/min; PreTx – Ctrl: −0.4 Cl (−3.2 to 2.3) μmol/kg/min]. During the hyperinsulinaemic clamp, endogenous glucose production was significantly less suppressed after transplantation [PostTx – PreTx: 2.4 Cl (0.1–4.8) μmol/kg/min] while similarly suppressed in controls and patients before transplantation [PreTx – Ctrl: 0.0 Cl (−5.1 to 5.1) μmol/kg/min]. Peripheral glucose uptake during the clamp was similar in patients before and after transplantation and in controls [PostTx – PreTx: −1.0 Cl (−4.5 to 6.5) μmol/kg/min; PreTx – Ctrl: −4.2 Cl (−11.6 to 3.1) μmol/kg/min] (Table 3 and Fig. 1b).

Insulin and glucagon

In the basal period, insulin concentration was similar in patients before and after transplantation and in controls [change from PreTx to PostTx: 22 Cl (−15 to 74)%; difference from Ctrl to PreTx: 58 Cl (−23 to 221)%]. During the hyperinsulinaemic clamp, the insulin concentrations were also comparable [PostTx – PreTx: −45.0 Cl (−119.9 to 29.9) pmol/l; PreTx – Ctrl: 74.4 Cl (−24.9 to 173.6) pmol/l] (Table 3).

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Table 2. Body composition and biochemical measures.

	PreTx	PostTx	Ctrl
Body composition			
Weight (kg)	77.1 (51.4–87.5)	81.6 (55.2–92.9)*	78.9 (54.4–95.5)
Total fat mass (kg)	20.4 (11.1–34.5)	26.1 (14.6–39.1)*	19.0 (7.1–49.1)
Fat percentage (%)	26.7 (14.6–41.6)	32.4 (20.1–42.7)*	23.3 (9.2–53.8)
Lean body mass (kg)	55.9 (34.6–67.6)	54.8 (34.1–67.0)	58.4 (42.8–69.8)
Trunk-to-limb fat ratio (%)	100.2 (77.1–134.1)	93.5 (60.1–126.6)	82.5 (50.0–133.3)
Biochemical			
Creatinine (μmol/l)	741 (533–1,320)	163 (106–374)*	77 (60–90)†
Glucose (mmol/l)	5.1 (4.4–6.5)	5.3 (4.6–6.0)	4.8 (4.0–5.4)
2-h glucose (mmol/l)	6.9 (4.8–8.7)	–	5.3 (3.8–7.5)†
HbA1c (%)	5.1 (4.4–5.5)	5.3 (4.9–5.8)*	5.2 (4.9–5.4)
HbA1c (mmol/mol)	32.7 (24.6–37.0)	34.9 (30.1–40.0)*	32.8 (30.0–36.0)
Cholesterol (mmol/l)	4.6 (2.3–6.2)	5.5 (3.8–8.7)	4.3 (3.6–4.9)
HDL (mmol/l)	1.2 (0.8–2.0)	1.2 (0.9–1.6)	1.5 (0.9–2.5)
LDL (mmol/l)	2.7 (1.5–4.4)	3.1 (2.1–6.0)	2.6 (1.6–3.0)
VLDL (mmol/l)	0.8 (0.0–2.4)	1.2 (0.2–5.1)	0.3 (0.0–0.7)
Triglycerides (mmol/l)	2.3 (0.7–6.2)	3.4 (1.3–12.8)	1.0 (0.6–2.6)

PreTx, before transplantation; PostTx, 6 months after transplantation; Ctrl, control subjects; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

Values expressed as mean (range). 2-h glucose is measured during the oral glucose tolerance test. All other biochemical results are fasting values.

*Significant difference between PreTx and PostTx ($P < 0.05$).

†Significant difference between PreTx and Ctrl ($P < 0.05$).

Table 3. Average values during clamp examination.

	PreTx	PostTx	Ctrl
Basal period			
Insulin (pmol/l)	66.8 (27.0–252.0)	81.4 (45.0–223.0)	42.4 (16.0–168.0)
Glucagon (pmol/l)	23.8 (11.7–65.9)	12.5 (4.4–35.1)*	8.3 (4.2–13.4)†
Glucose Ra_{Endo} (μmol/kg/min)	12.8 (10.1–14.7)	13.3 (10.2–14.5)	13.2 (9.3–15.6)
Glycerol Ra (μmol/kg/min)	2.6 (1.5–5.3)	4.3 (2.1–8.6)	3.2 (1.9–6.9)
Glycerol (μmol/l)	62 (37–109)	94 (57–221)*	83 (54–227)
Clamp period			
Glucose (mmol/l)	4.9 (4.7–5.3)	5.1 (4.7–5.5)	5.0 (4.7–5.3)
GIR (μmol/kg/min)	15.1 (9.1–23.7)	9.8 (2.8–14.6)*	20.2 (9.9–33.7)
Insulin (pmol/l)	319 (135–536)	274 (199–389)	244 (164–364)
Glucagon (pmol/l)	12.7 (5.4–27.7)	7.6 (2.3–32.3)*	2.9 (0.7–7.1)†
Glucose Ra_{Endo} (μmol/kg/min)	7.0 (4.8–8.5)	9.4 (7.4–11.8)*	7.0 (−3.8 to 10.1)
Glucose Rd (μmol/kg/min)	18.1 (12.9–24.5)	17.1 (12.2–22.7)	22.3 (14.6–34.3)
Glycerol Ra (μmol/kg/min)	1.1 (0.8–1.3)	2.0 (1.0–3.7)*	1.1 (0.8–2.5)
Glycerol (μmol/l)	32 (18–62)	45 (20–99)	33 (16–112)

PreTx, before transplantation; PostTx, 6 months after transplantation; Ctrl, control subjects; GIR, glucose infusion rate; Glucose Ra_{Endo}, rate of glucose appearance; Glucose Rd, rate of glucose disappearance; Glycerol Ra, rate of glycerol appearance.

Glucose Ra_{Endo} ($N = 6$), glucose Rd ($N = 6$) and glycerol Ra ($N = 5$) measured in a subset of the participants. Values expressed as mean or geometric mean (range).

*Significant difference between PreTx and PostTx ($P < 0.05$).

†Significant difference between PreTx and Ctrl ($P < 0.05$).

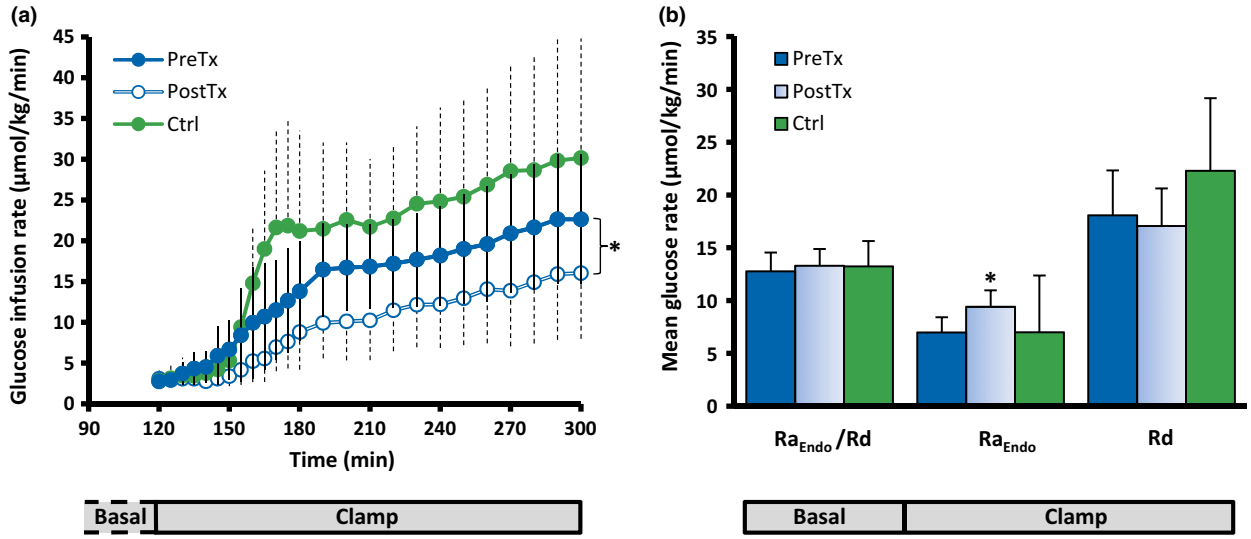

Figure 1 (a) Glucose infusion rate during hyperinsulinaemic–euglycaemic clamp in all participants before transplantation (PreTx), after transplantation (PostTx) and in controls (Ctrl) with standard deviation. Similar measurement points in time are achieved with linear interpolation. (b) Mean endogenous glucose production/peripheral glucose uptake in six patients and controls during basal and clamp measured by glucose rate of appearance (Ra_{Endo})/disappearance (Rd) with standard deviation. *Significant difference between PreTx and PostTx.

In the basal period, glucagon concentration was significantly lower after transplantation [change from PreTx to PostTx: −47 Cl (−69 to −10)%] and in controls [difference from Ctrl to PreTx: 187 Cl (77–366)%]. During the hyperinsulinaemic clamp, glucagon was suppressed to a significantly lower concentration after transplantation [change from PreTx to PostTx: −40 Cl (−61 to −8)%] and in controls [difference from Ctrl to PreTx: 341 Cl (135–729)%] (Table 3).

Whole-body lipolysis

In the basal period, whole-body lipolysis measured by glycerol rate of appearance was similar in patients before and after transplantation and in controls [change from PreTx to PostTx: 63 Cl (−9 to 192)%; difference from Ctrl to PreTx: −17 Cl (−61 to 75)%]. During the hyperinsulinaemic clamp, whole-body lipolysis was significantly less suppressed after transplantation [change from PreTx to PostTx: 75 Cl (3–196)%] while similarly suppressed in controls and patients before transplantation [difference from Ctrl to PreTx: −1 Cl (−42 to 69)%] (Table 3).

In the basal period, glycerol concentration was significantly higher after transplantation [change from PreTx to PostTx: 50 Cl (20–86)%] with similar concentrations in the controls and the patients before transplantation [difference from Ctrl to PreTx: −25 Cl (−53 to 20)%]. During the hyperinsulinaemic clamp, glycerol was suppressed to similar concentrations in patients before and after transplantation and in controls [change from PreTx to PostTx: 75 Cl (−7 to 103)%; difference from Ctrl to PreTx: −2 Cl (−49 to 57)%] (Table 3).

Body composition by DXA

Six months after transplantation, patients significantly gained weight [PostTx – PreTx: 4.5 Cl (0.6–8.5) kg] with increased total fat mass [PostTx – PreTx: 5.7 Cl (1.6–9.8) kg] and fat percentage [PostTx – PreTx: 5.7 Cl (1.9–9.6) percentage point] and similar lean body mass [PostTx – PreTx: −1.1 Cl (−3.4 to 1.2) kg]. Prior to transplantation, patients had similar body composition as controls (Table 2).

Follow-up

After a mean follow-up period of 5.9 years (range 3.4–9.0) from transplantation, one patient had chronic graft failure treated with dialysis and the remaining eight patients had a functioning graft with mean eGFR 45 ml/min/1.73 m^2 (range 26–78). One 32-year-old Caucasian male with no family disposition to diabetes and treated with cyclosporine developed PTDM 8 years after transplantation. This patient had a normal fasting glucose and glucose tolerance but elevated fasting levels of insulin, triglycerides and a relative low insulin sensitivity (average glucose infusion rate 10.3 µmol/kg/min) before transplantation. The patient had a marginal weight gain (1.1 kg) 6 months after transplantation.

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Discussion

We show that insulin sensitivity, as measured by the hyperinsulinaemic–euglycaemic clamp, is reduced 6 months after kidney transplantation and characterized by an impaired suppression of the endogenous glucose production and whole-body lipolysis, whereas peripheral glucose uptake was unaffected.

Determining alterations in insulin sensitivity after kidney transplantation is challenging. The renal clearance of glucoregulatory hormones and the metabolic activity such as gluconeogenesis of both the diseased kidneys as well as the transplanted kidney graft is difficult to predict. Models using the assumption of an unchanged insulin action or a specific dose–response curve between glucose and insulin are therefore biased. Moreover, several indices of insulin sensitivity have reduced prognostic value when used in longitudinal studies [20] and hence not suitable to measure development of insulin resistance.

Therefore, in the present study, we used the hyperinsulinaemic–euglycaemic clamp method as described by DeFronzo *et al.* [21] which offers a more direct measure of the insulin sensitivity as it minimizes the variation of glucose and glucoregulatory hormones and thereby bypassing the dynamic feedback loop of glucose and insulin. The method does not assume any specific dose–response curve (linear or nonlinear) between glucose and insulin and has been proven to be reproducible [21,22]. To our knowledge, the present study is the first to investigate insulin sensitivity using the hyperinsulinaemic–euglycaemic clamp in a longitudinal study of kidney transplant patients.

Our finding of a marked decrease in insulin sensitivity as measured by the hyperinsulinaemic–euglycaemic clamp following kidney transplantation is in accordance with some but not all previous studies. In a cross-sectional study using the hyperinsulinaemic–euglycaemic clamp with glucose tracers, Ekstrand *et al.* [7] found that the peripheral glucose uptake in kidney transplanted patients was lower than seen in healthy controls, while the endogenous glucose production was similar. The results were similar whether the patients had normal glucose tolerance or PTDM. In contrast, we did not find a significant decrease in peripheral glucose uptake but a significant impaired suppression of the endogenous glucose production during the hyperinsulinaemic clamp. On an individual patient's level though, five of the six patients with glucose tracer data exhibited a decrease in peripheral glucose uptake. Therefore, we cannot exclude a reduced insulin sensitivity on peripheral glucose uptake after transplantation. Moreover, in the study by Ekstrand *et al.*, the endogenous glucose production was almost completely suppressed in both the patients and the controls which together with the cross-sectional nature of their study may explain why they did not detect an impaired suppression of the endogenous glucose production after transplantation.

Longitudinal studies, using a wide variety of insulin sensitivity indices, have indicated a decreased [10,11,23], unchanged [9] and even improved [8,24] insulin sensitivity following kidney transplantation. Unfortunately, none of these indices differentiates between insulin sensitivity on endogenous glucose production and peripheral glucose uptake although indices based on fasting glucose and insulin concentrations may reflect the former to a greater extend [25]. Several indices, including the homoeostatic model assessment (HOMA) and insulin sensitivity indices from oral glucose tolerance test, have been compared to the hyperinsulinaemic–euglycaemic clamp in transplanted patients yielding correlation coefficients between 0.4 and 0.6 [26,27]. This moderate association between indices and clamp derived measurements which decrease further in a longitudinally setting [20] may explain the heterogeneous findings of the effect of kidney transplantation on insulin sensitivity in the previous studies.

Elevated glucagon concentrations are commonly observed in end-stage renal disease and have been attributed to a reduced metabolic clearance rate [28], although some assays may include other glucagon-like immunoreactive compounds present to a larger extent in patients with uraemia [29]. In the present study, we confirmed the hyperglucagonaemia in the fasting state as well as during the clamp using a glucagon assay validated in patients with end-stage renal disease [17]. After transplantation, both the fasting and insulin-suppressed glucagon levels decreased which may be due to an increased metabolic clearance rate caused by the transplanted kidney. The endogenous glucose production in the fasting state is expected to be affected by glucagon concentrations, but interestingly, we did not find any difference between the three groups. This could suggest a hepatic glucagon resistance induced by uraemia and partly alleviated by the transplantation as the endogenous glucose production was unchanged after transplantation in spite of nearly halved glucagon concentrations. Further insights into the effect of glucagon following transplantation are warranted.

The impact of kidney transplantation on insulins ability to control adipose tissue breakdown of triglycerides into glycerol and free fatty acids (lipolysis) has

been sparsely investigated. We find that kidney transplanted patients develop a reduced effect of insulin to inhibit lipolysis as shown by the impaired suppression of glycerol rate of appearance during the hyperinsulinaemic–euglycaemic clamp. This is in accordance with the results of Boden *et al.* [30] who furthermore found similar fatty acid oxidation compared to healthy controls in a cross-sectional study including six kidney transplanted patients. In the basal period, we also found elevated glycerol concentrations after transplantation indicating an increased lipolysis in the fasting state as well. In accordance, Ekstrand *et al.* [7] found elevated fasting levels of free fatty acids in patients who developed diabetes after transplantation, but they did not detect a difference in the effect of insulin on free fatty acid concentration which may be explained by their relative high dose of insulin infusion which exceeded twice the amount administered in the present study. The causal relationship of a reduced effect of insulin in adipose tissue is uncertain though as an excess availability of free fatty acids also reduces insulin sensitivity [31]. Further studies should focus on the possible association between lipolysis and insulin sensitivity following transplantation.

One of the main factors in the pathogenesis of PTDM is believed to be the mandatory use of immunosuppressive drugs which have shown multiple diabetogenic adverse effects. In experimental settings, glucocorticoids induce both peripheral and central insulin resistance [32], inhibit glucose mediated insulin secretion [33] and decrease insulin-mediated suppression of lipolysis [34]. Likewise, longer treatment with calcineurin inhibitors as well as mTOR inhibitors may also induce insulin resistance [35], inhibit insulin secretion [36] and induce lipolysis [37]. This is in agreement with clinical studies where insulin resistance, impaired insulin secretion and dyslipidaemia are associated with the extent of immunosuppressive treatment used [23,38,39].

Another known cause of insulin resistance is the uraemic intoxication itself. Although the precise mechanism is uncertain, insulin resistance is observed in even mild renal impairment [40] and dialysis seems to attenuate the resistance to some extent [41]. After transplantation, the uraemic intoxication is alleviated, and hence, insulin sensitivity should increase, but graft failure by any cause such as delayed graft function, rejection or infection could diminish this beneficial effect on insulin sensitivity.

Our results confirm the development of insulin resistance after transplantation characterized by higher lipolysis and adipose weight gain which are all associated with the development of diabetes. To reduce the risk of PTDM, clinicians should minimize the use of immunosuppressive drugs while maintaining an optimal graft function and avoid weight gain in the patients.

There are several limitations in this study. Firstly, the size of the studied population is small and some results were obtained in a subset. Although small sample sizes are common in clinical experiments, some caution should be taken when extrapolating the results to the general population of kidney transplanted patients. Secondly, one of the premises of the clamp technique is the achievement of similar insulin levels by constant insulin infusion. As insulin is metabolized in the kidneys [13], the plasma concentration during the clamp could be affected by a change in insulin clearance due to the kidney transplantation itself. In the present study, we observed that insulin levels during the clamp were slightly numerical (not significant) higher before transplantation; however, any significant effect on the glucose utilization to these small differences in insulin concentrations is not expected.

Conclusions

We find that insulin sensitivity, as measured by the hyperinsulinaemic–euglycaemic clamp technique, is reduced 6 months after kidney transplantation. The suppression of endogenous glucose production and lipolysis during the clamp was impaired after transplantation, while insulin-mediated peripheral glucose uptake remained stable. These findings suggest that a reduced insulin sensitivity mainly based on suppression of endogenous glucose production and lipolysis could be significant contributors to the development of PTDM.

Authorship

MBJ: screened and included participants, performed examinations, data analyses and wrote the initial draft of the manuscript. MH: designed the study, screened and included participants and performed examinations. GVH: designed the study and performed data analyses. CB and JMH: designed the study, screened and included participants. ERM and BFR: designed the study. All authors contributed to the interpretation of the data and the revision of the manuscript and approved the final version of the manuscript.

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Conflict of Interest

The authors have no relevant conflict of interest to disclose. The results presented in this study have not been published previously in whole or part, except in abstract format.

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REFERENCES

1. Bergrem HA, Valderhaug TG, Hartmann A, *et al.* Undiagnosed diabetes in kidney transplant candidates: a case-finding strategy. *Clin J Am Soc Nephrol* 2010; **5**: 616.
2. DeFronzo RA. Pathogenesis of glucose intolerance in uremia. *Metabolism* 1978; **27**: 1866.
3. Schmitz O. Peripheral and hepatic resistance to insulin and hepatic resistance to glucagon in uraemic subjects. Studies at physiologic and supraphysiologic hormone levels. *Acta Endocrinol (Copenh)* 1988; **118**: 125.
4. Smith D, DeFronzo RA. Insulin resistance in uremia mediated by postbinding defects. *Kidney Int* 1982; **22**: 54.
5. Hornum M, Lindahl JP, Von Zur-Mühlen B, Jenssen T, Feldt-Rasmussen B. Diagnosis, management and treatment of glucometabolic disorders emerging after kidney transplantation: a position statement from the Nordic Transplantation Societies. *Transpl Int* 2013; **26**: 1049.
6. Hjelmesaeth J, Hagen M, Hartmann A, Midtvedt K, Egeland T, Jenssen T. The impact of impaired insulin release and insulin resistance on glucose intolerance after renal transplantation. *Clin Transplant* 2002; **16**: 389.
7. Ekstrand AV, Eriksson JG, Grönhagen-Riska C, Ahonen PJ, Groop LC. Insulin resistance and insulin deficiency in the pathogenesis of posttransplantation diabetes in man. *Transplantation* 1992; **53**: 563.
8. Nam JH, Mun JI, Kim SI, *et al.* beta-Cell dysfunction rather than insulin resistance is the main contributing factor for the development of postrenal transplantation diabetes mellitus. *Transplantation* 2001; **71**: 1417.
9. Hecking M, Haidinger M, Doller D, *et al.* Early basal insulin therapy decreases new-onset diabetes after renal transplantation. *J Am Soc Nephrol* 2012; **23**: 739.
10. Hur KY, Kim MS, Kim YS, *et al.* Risk factors associated with the onset and progression of posttransplantation diabetes in renal allograft recipients. *Diabetes Care* 2007; **30**: 609.
11. Hornum M, Jørgensen KA, Hansen JM, *et al.* New-onset diabetes mellitus after kidney transplantation in Denmark. *Clin J Am Soc Nephrol* 2010; **5**: 709.
12. Porrini E, Moreno JM, Osuna A, *et al.* Prediabetes in patients receiving tacrolimus in the first year after kidney transplantation: a prospective and multicenter study. *Transplantation* 2008; **85**: 1133.
13. Corvilain J, Brauman H, Delcroix C, Toussaint C, Vereerstraeten P, Franckson JR. Labeled insulin catabolism in chronic renal failure and in the anephric state. *Diabetes* 1971; **20**: 467.
14. Bergrem HA, Valderhaug TG, Hartmann A, Bergrem H, Hjelmesaeth J, Jenssen T. Glucose tolerance before and after renal transplantation. *Nephrol Dial Transplant* 2010; **25**: 985.
15. Alberti KGMM, Zimmet PZ. Definition, diagnosis and 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.
16. Bornø A, Foged L, van Hall G. Glucose and glycerol concentrations and their tracer enrichment measurements using liquid chromatography tandem mass spectrometry. *J Mass Spectrom* 2014; **49**: 980.
17. Wewer Albrechtsen NJ, Hartmann B, Veedfald S, *et al.* Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia* 2014; **57**: 1919.
18. Wolfe RR, Chinkes DL. *Isotope Tracers in Metabolic Research. Principles and Practice of Kinetic Analysis*, 2nd edn. Hoboken: Wiley, 1992.
19. Beylot M, Martin C, Beaufrere B, Riou JP, Mornex R. Determination of steady state and nonsteady-state glycerol kinetics in humans using deuterium-labeled tracer. *J Lipid Res* 1987; **28**: 414.
20. Xiang AH, Watanabe RM, Buchanan TA. HOMA and Matsuda indices of insulin sensitivity: poor correlation with minimal model-based estimates of insulin sensitivity in longitudinal settings. *Diabetologia* 2014; **57**: 334.
21. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol* 1979; **237**: E214.
22. Le DSNT, Brookshire T, Krakoff J, Bunt JC. Repeatability and reproducibility of the hyperinsulinemic-euglycemic clamp and the tracer dilution technique in a controlled inpatient setting. *Metabolism* 2010; **58**: 304.
23. Asberg A, Midtvedt K, Voytovich MH, *et al.* Calcineurin inhibitor effects on glucose metabolism and endothelial function following renal transplantation. *Clin Transplant* 2009; **23**: 511.
24. Mericq V, Salas P, Pinto V, *et al.* Steroid withdrawal in pediatric kidney transplant allows better growth, lipids and body composition: a randomized controlled trial. *Horm Res Paediatr* 2013; **79**: 88.
25. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 1999; **22**: 1462.
26. Hjelmesæth J, Midtvedt K, Jenssen T, Hartmann A. Insulin resistance after renal transplantation: impact of immunosuppressive and antihypertensive therapy. *Diabetes Care* 2001; **24**: 2121.
27. Oterdoom LH, De Vries APJ, Van Son WJ, *et al.* Validation of insulin resistance indexes in a stable renal transplant population. *Diabetes Care* 2005; **28**: 2424.
28. Sherwin RS, Bastl C, Finkelstein FO, *et al.* Influence of uremia and hemodialysis on the turnover and metabolic effects of glucagon. *J Clin Invest* 1976; **57**: 722.
29. Baldissera FG, Holst JJ. Glicentin 1-61 probably represents a major fraction of glucagon-related peptides in plasma of anaesthetized uraemic pigs. *Diabetologia* 1986; **29**: 462.
30. Boden G, Chen X, Ruiz J, Heifets M, Morris M, Badosa F. Insulin receptor down-regulation and impaired antilipolytic action of insulin in diabetic patients after pancreas/kidney transplantation. *J Clin Endocrinol Metab* 1994; **78**: 657.

31. Roden M, Price TB, Perseghin G, *et al.* Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest* 1996; **97**: 2859.
32. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. *J Clin Endocrinol Metab* 1982; **54**: 131.
33. Delaunay F, Khan A, Cintra A, *et al.* Pancreatic β cells are important targets for the diabetogenic effects of glucocorticoids. *J Clin Invest* 1997; **100**: 2094.
34. van Raalte DH, Brands M, van der Zijl NJ, *et al.* Low-dose glucocorticoid treatment affects multiple aspects of intermediary metabolism in healthy humans: a randomised controlled trial. *Diabetologia* 2011; **54**: 2103.
35. Lopes PC, Fuhrmann A, Carvalho F, *et al.* Cyclosporine A enhances gluconeogenesis while sirolimus impairs insulin signaling in peripheral tissues after 3 weeks of treatment. *Biochem Pharmacol* 2014; **91**: 61.
36. Øzbay LA, Smidt K, Mortensen DM, Carstens J, Jørgensen KA, Rungby J. Cyclosporin and tacrolimus impair insulin secretion and transcriptional regulation in INS-1E beta-cells. *Br J Pharmacol* 2011; **162**: 136.
37. Pereira MJ, Palming J, Rizell M, *et al.* The immunosuppressive agents rapamycin, cyclosporin A and tacrolimus increase lipolysis, inhibit lipid storage and alter expression of genes involved in lipid metabolism in human adipose tissue. *Mol Cell Endocrinol* 2013; **365**: 260.
38. Boots JMM, van Duijnhoven EM, Christiaans MHL, Wolffenbuttel BHR, van Hooff JP. Glucose metabolism in renal transplant recipients on tacrolimus: the effect of steroid withdrawal and tacrolimus trough level reduction. *J Am Soc Nephrol* 2002; **13**: 221.
39. Hjelmesaeth J, Hartmann A, Kofstad J, Egeland T, Stenstrøm J, Fauchald P. Tapering off prednisolone and cyclosporin the first year after renal transplantation: the effect on glucose tolerance. *Nephrol Dial Transplant* 2001; **16**: 829.
40. Becker B, Kronenberg F, Kielstein JT, *et al.* Renal insulin resistance syndrome, adiponectin and cardiovascular events in patients with kidney disease: the mild and moderate kidney disease study. *J Am Soc Nephrol* 2005; **16**: 1091.
41. DeFronzo RA, Tobin JD, Rowe JW, Andres R. Glucose intolerance in uremia. Quantification of pancreatic beta cell sensitivity to glucose and tissue sensitivity to insulin. *J Clin Invest* 1978; **62**: 425.