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Plasma adiponectin before and after kidney transplantation

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Summary

The role of plasma adiponectin (ADPN) in patients with impaired kidney function and following kidney transplantation (Tx) is debated. We aimed to: (i) determine whether pretransplant ADPN level is an independent risk factor for deterioration of glucose tolerance including development of new-onset diabetes mellitus after Tx, (ii) describe which parameters that influence the ADPN concentration before and after Tx. Fifty-seven nondiabetic kidney allograft recipients and 40 nondiabetic uraemic patients were included. The Tx group was examined at baseline and 3 and 12 months after Tx. The uraemic control group was examined twice, separated by 12 months. ADPN levels declined significantly following Tx ($P < 0.0001$), while estimated glomerular filtration rate (eGFR) increased ($P < 0.0005$). eGFR, BMI and insulin sensitivity index were independently associated with ADPN in a multivariate regression analysis, whereas an ordinal logistic regression analysis revealed no predictive characteristic of ADPN for aggravation of the glucose tolerance after Tx. In conclusion, kidney transplantation is accompanied by a significant reduction in ADPN concentration. Several factors determine the ADPN concentration before and after Tx including kidney function, insulin resistance, use of immunosuppressive agents and BMI. Pretransplant ADPN level did not predict development of new-onset diabetes mellitus or even deterioration of the glucose tolerance following Tx.

Introduction

Adiponectin (ADPN) is an adipocyt-derived 30 kDa glycoprotein that circulates in plasma and amounts approximately 0.01% of total plasma proteins. It has multiple functions including insulin-sensitizing, anti-atherogenic and anti-inflammatory properties [1,2]. A high plasma concentration of ADPN has been shown to protect against development of type 2 diabetes (T2D) in healthy individuals [3], whereas low ADPN levels are associated with enhanced risk of insulin resistance (IR) [2,4,5], T2D [5,6], obesity [5–8] and cardiovascular disease (CVD) [1,6,9,10] in subjects with normal kidney function.

The role of ADPN in patients with impaired kidney function are, however, questionable and reports concerning the association between ADPN and IR, body mass index (BMI), and T2D in patients with end-stage renal disease (ESRD) and following kidney transplantation (Tx), are conflicting. Despite increased prevalence of IR, CVD and atherosclerosis in patients with ESRD, ADPN levels are 2–3 times higher than in matched healthy subjects [11]. This observation challenges the concept that

high plasma concentrations of ADPN protect against CVD and T2D, and suggests that the kidneys play a role in the elimination of ADPN. A high level of ADPN is, however, inversely related to cardiovascular events in patients with varying degree of renal impairment [11–13] and to BMI in patients with ESRD [11], indicating that ADPN may have some of the same predictive characteristics in patients with ESRD, as in subjects with normal kidney function. Furthermore, animal models suggest protective effects of ADPN in regard to albuminuria and renal fibrosis and it is well established that ADPN levels are directly correlated to overt proteinuria, which again supports the presence of a link between ADPN and the kidneys [14]. Little is known about the mechanism(s) of ADPN metabolism and controversy exists whether the ADPN level is increased in renal disease as a result of low glomerular filtration rate (GFR) [15,16] or in response to chronic inflammation and/or the cytokine activation present in uraemia [17–19]. A few studies have investigated the change in ADPN concentration following successful Tx. One found a significant reduction in ADPN concentration after Tx [20], whereas another demonstrated unaltered levels before and after Tx [21]. However, the transplanted patients still had significantly higher ADPN levels compared with a healthy control group, despite a normal kidney allograft function had been obtained [21,22]. Few studies have examined ADPN in relation to change in glucose tolerance and development of new-onset diabetes mellitus (NODM) after Tx. In two studies, low pretransplant concentration of ADPN was shown to be an independent risk factor for development of NODM [23,24], whereas in a third study, no such correlation was found [25]. In this study, we investigated the potential predictive character of pretransplant ADPN levels in relation to aggravation of glucose tolerance including development of NODM following Tx. Furthermore, the relation between ADPN concentration and kidney function, cardiovascular risk factors, and glucometabolic parameters before and after Tx were evaluated.

Patients and methods

The study design and procedure was previously described [26].

Design

We performed a prospective, observational, national multicentre study including 54 (67%) of 81 patients, primarily Caucasians, with scheduled living donor Tx in the period between January 2006 and March 2008 at four Danish transplantation centres: Copenhagen, Aarhus, Odense and Herlev University Hospitals (Tx group). All 81 patient charts were prescreened, and the following criteria caused exclusion: Diabetes ($n = 10$), prednisolone treatment >12.5 mg/day ($n = 3$), >1 previous Tx ($n = 0$) and newly initiated high-dose immunosuppressive treatment as in AB0-incompatible Tx ($n = 4$). Ten patients did not want to participate, leaving us with 54 patients in the Tx group at baseline. A control group consisted initially of 52 patients from the waiting list for Tx and with allegiance to the Department of Nephrology at Copenhagen and Herlev University Hospitals. Criteria for screening in both groups were: Age <65 years and eligibility for Tx. During the follow-up period, three patients from the control group underwent Tx with a deceased donor and were included in the Tx group. In the control group, four patients died and five patients did not want to participate in the 12-month examination, leaving us with a total of 57 patients in the Tx group and 40 patients in the uraemic control group who completed the study (demographic data are presented in Table 1). Patients in the Tx group were examined at baseline and 3 and 12 months following Tx. Patients in the uraemic control group were examined twice, separated by 12 months (Time 0 and 12 months). The regional ethics committee (KF 01279825) and The Data Protection Agency (2006-41-5640) approved the study. All participants gave their informed written consent. The study was conducted according to the Helsinki Declaration.

Table 1. Baseline demographic and clinical data.

	Tx group $n = 57$	Control group $n = 40$
Age (years)	39 ± 13*	47 ± 11
Gender (male/female)	38/19	27/13
ESRD duration (months)	24 (0–134)**	45 (1–168)
Ever smoking, *n* (%)	27 (47)	26 (65)
First-degree relative with DM, *n* (%)	2 (4)	4 (10)
Renal diagnose, *n* (%)		
Glomerulonephritis	28 (49)	13 (33)
Hypertensive kidney disease	5 (9)	11 (28)
Vasculitis	1 (2)	1 (3)
PKD	3 (5)	7 (18)
Other/unknown	20 (35)	8 (20)
Former Tx	11 (19)	10 (25)
Dialysis, *n* (%)		
HD	28 (49)	28 (70)
CAPD	19 (33)	9 (27)
Predialysis	10 (18)	3 (8)

Tx, kidney transplantation; ESRD, end-stage renal disease; PKD, polycystic kidney disease; HD, haemodialysis; CAPD, continuous ambulatory peritoneal dialysis.

Data are presented as mean ± SD, median (range), numbers (*n*) and per cent (%).

Wilcoxon rank sum test or two-sample *t*-test used where appropriate to test: Tx group vs. control group: $*P < 0.05$, $**P < 0.005$.

Study procedure

At each visit, the participants met after an overnight fast including abstinence for coffee, tobacco and exercise for 10 h. Blood samples were drawn for determination of plasma values of ADPN, fasting plasma glucose (FPG), insulin, HbA1c, creatinine, von Willebrand Factor (vWF), total cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides, C-reactive protein (CRP) and uric acid. Blood pressure, height, body weight and waist- and hip-circumferences were measured. A 75-g oral glucose tolerance test (OGTT) was performed for determination of glucose tolerance and IR. Haemodialysis patients were examined between the days of dialysis. Peritoneal dialysis (PD) patients had the last peritoneal fluid installed at 10 PM the day before examination and were drained in the morning at 6 AM to minimize bias from glucose absorbed from the peritoneum. Examinations began between 8 and 11 AM in all participants, however, with the OGTT done at least 4 h after draining the peritoneal fluid in PD patients. Fasting blood samples were drawn from an antecubital vein.

Analyses

Plasma ADPN was measured in duplicate using a validated in-house Time-Resolved Immuno-Flourometric Assay (TRIFMA) [27] based on two monoclonal antibodies and recombinant human ADPN (R&D Systems, Abingdon, UK). Samples were analysed with a detection limit of 1.5 μg/l and an intraassay coefficient of variance (CV) <5% and interassay CV <7%. The ADPN molecule is known to form a wide range of polymers of which the predominant polymers include trimers, hexamers and highly congregated multimers [28]. Previous experiments, according to Laemmli's method [29], have demonstrated that both monoclonal antibodies used are able to detect several ADPN polymers in plasma, including the three major molecular forms mentioned. Plasma glucose (PG) concentration was analysed by the glucose-hexokinase method (Gluco-quant®; Roche Diagnostics GmbH, D-68298 Mannheim, Germany), insulin was measured using enzyme-linked immunosorbent assay kits (ELISA) (Elecsys; Roche Diagnostics), and vWF was determined using the ELISA method (Elisa reader ANTHOS ht 2; Labtech Instrument 12550; Triolab AS, Denmark). All assays were automated and performed on a Cobas Fara robot (Roche Diagnostics). Standard laboratory methods were applied for the analysis of the remaining parameters. Estimated glomerular filtration rate (eGFR) was calculated using the Cockcroft-Gault formula [30]. <15 ml/min was used as an estimate of eGFR in the Tx group at baseline and in the control group at 0 and 12 months, as calculation of eGFR is not validated in this group of patients [31].

Evaluation of glucose tolerance and IR

Glucose tolerance was evaluated according to the ADA 2007 guidelines [32]. PG and insulin were measured at time points −30, −15, 0, 30, 60, 90 and 120 min during the 2-h OGTT and the insulin sensitivity index (ISI) was calculated as 10 000/square root of [fasting glucose × fasting insulin] × [mean glucose × mean insulin during OGTT] according to Matsuda *et al.* [33] (Table 2). ISI is a marker of IR.

Immunosuppressive treatment

Immunosuppression in the Tx group varied to some extent between the centres.

Corticosteroids

The majority of patients received 100–500 mg of intravenous methylprednisolone preoperatively. Post-Tx treatment with oral prednisolone was started by 20–100 mg/day and tapered to a dose of 7.5–10 mg at 3 months and to 5–7.5 mg at 9–12 months. In the seven patients from Odense University Hospital, no prednisolone treatment was started in concordance with the routine treatment regimen. Rejection episodes in all hospital regimens were treated with intravenous methylprednisolone, 500 mg, for 3–5 days, followed by oral prednisolone.

Calcineurin inhibitors

Forty-three patients started on cyclosporine (Sandimmune Neoral®; Novartis Healthcare A/S, Copenhagen, Denmark) at a dose of 2.5–6 mg/kg BID tapered to a trough level of whole-blood concentration of 150–300 μg/l for the first 3 months and 100–150 μg/l thereafter. The remaining 14 patients started on tacrolimus (Prograf®; Astellas Pharma a/s, Kastrup, Denmark) at a dose of 0.075–0.15 mg/kg BID tapered to a whole-blood concentration of 8–15 μg/l for the first 3 months and 5–10 μg/l thereafter. A few patients ($n = 8$) changed from one treatment modality to another during the study, and two patients were changed from a calcineurin inhibitor to sirolimus (Rapamune®; Wyeth, Glostrup, Denmark) treatment (Table 2).

Statistical analyses

Data analyses were done using Statistical Analysis Software (SAS®, SAS Institute Inc., Cary, NC, USA) version 9.1. Unless specified otherwise, continuous data are described as mean ± SD for normal distributions, and median and range for skewed distributions. Paired data within groups were compared using *t*-tests for normally distributed data. Group comparisons of continuous data were done using two-sample *t*-test for normally distributed data and

Table 2. Clinical data.

	Tx group n = 57			Control group n = 40	
Time (months)	0	3	12	0	12
Laboratory results					
Plasma ADPN (mg/l)	16.8 ± 8.3	10.4 ± 4.5***	10.1 ± 4.3***	14.9 ± 6.4	14.9 ± 6.7
ΔADPN (mg/l)		−4.9 (−26.4–5.4)***	−5.0 (−37.2–4.1)***		0.42 (−13.8–16.1)
Plasma creatinine (μmol/l)	867 ± 364	132 ± 40***	127 ± 36***	801 ± 277	824 ± 215
eGFR (ml/min)	<15†	73.6 ± 20.3***	78.0 ± 22.7***	<15†	<15†
ΔeGRF (ml/min)		66.7 ± 21.6***	62.2 ± 19.7***		0
Plasma vWF (kIU/l)	1.83 ± 0.79	1.97 ± 0.97	1.56 ± 0.63	1.65 ± 0.78	1.69 ± 0.72
Plasma CRP (mg/l)	3.60 ± 3.42	3.85 ± 3.25	4.92 ± 4.56	3.97 ± 6.46	4.72 ± 5.62
Plasma uric acid (mmol/l)	0.36 ± 0.10	0.45 ± 0.13***	0.42 ± 0.13*	0.29 ± 0.10	0.29 ± 0.08
Total plasma cholesterol (mmol/l)	5.0 ± 1.3	5.9 ± 1.7**	5.1 ± 1.2	4.4 ± 1.1	4.3 ± 1.2
Plasma HDL-cholesterol (mmol/l)	1.4 ± 0.5	1.3 ± 0.3	1.3 ± 0.4	1.4 ± 0.4	1.3 ± 0.4
Plasma triglyceride (mmol/l)	2.0 ± 1.5	2.5 ± 1.3**	2.1 ± 1.5	1.3 ± 0.7	1.5 ± 0.7
Clinical data					
BMI (kg/m^2)	24.4 ± 3.9	25.4 ± 3.9*	26.3 ± 4.6*	24.1 ± 3.0	24.0 ± 2.7
Waist–hip ratio	0.92 ± 0.09	0.94 ± 0.09	0.93 ± 0.09	0.92 ± 0.08	0.91 ± 0.10
MAP (mmHg)	104 ± 14	97 ± 11*	98 ± 15*	102 ± 16	102 ± 14
AH	3 ± 1	2 ± 1	2 ± 1*	2 ± 1	2 ± 1
Glucose metabolism					
ISI	6.8 ± 3.9	4.9 ± 3.9**	4.9 ± 2.8**	7.9 ± 5.1	8.5 ± 4.9
FPG (mmol/l)	5.1 ± 0.5	5.6 ± 0.7***	5.5 ± 0.7***	5.1 ± 0.5	4.9 ± 0.9
OGTT $_{PG\ 120\ min}$ (mmol/l)	7.4 ± 1.6	8.7 ± 3.1	7.8 ± 2.5	7.5 ± 1.7	7.8 ± 2.4
HbA1c (%)	5.2 ± 0.4	5.6 ± 0.5***	5.6 ± 0.4***	5.2 ± 0.4	5.3 ± 0.4
DM, *n* (%)	0 (0)	11 (19)	8 (14)	0	2 (5)
IFG/IGT, *n* (%)	0/19 (33)	3/17 (35)	1/16 (30)	2/18 (50)	2/14 (40)
NGT, *n* (%)	38 (67)	26 (46)	32 (56)	20 (50)	22 (55)
Immunosuppressive treatment					
Prednisolone, *n* (%)	12 (21)	52 (91)	52 (91)	5 (2)	5 (2)
Prednisolone, mg	6	13	7	5	5
Tacrolimus, *n* (%)	1 (2)	19 (33)	21 (37)	0	0
Tacrolimus, mg	0	2 (4)	2 (3)		
Cyclosporine, *n* (%)	1 (2)	36 (63)	32 (56)	0	0
Cyclosporine, mg	0	214 (187)	145 (139)	0	0
Azathioprine/Sirolimus, *n* (%)	0	2 (4)	3 (5)	0	0
MMF (Cellcept), *n* (%)	4 (7)	52 (91)	46 (81)	0	0
MMF (Myfortic), *n* (%)	0	2 (4)	7 (15)	0	0

ADPN, adiponectin; AH, mean number of antihypertensives; AUC, area under the curve; BMI, body mass index; CMV, cytomegalovirus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; ISI, insulin sensitivity index; MAP, mean arterial pressure; MMF, mycophenolate mofetil; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; Tx, kidney transplantation; vWF, von willebrand factor; WHR, waist–hip ratio.

Data are presented as mean ± SD, median (range), numbers (*n*) and per cent (%).

Wilcoxon rank sum test or paired *t*-test used where appropriate to test: Tx patients before vs. after Tx: $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$. No significant differences in the control group between 0 and 12 months.

†<15 ml/min has been used as an estimate of eGFR in the ESRD patients, as calculation of eGFR is not validated in this group of patients [31].

Wilcoxon rank sum test for nonnormal distributed data. The distribution of data was assessed by graphical evaluation. Chi-squared or Fisher exact tests were used for group comparisons between categorical data. Correlation between ADPN and additional parameters were tested using Spearman's rank correlation coefficient and Kruskal–Wallis one-way analysis of variance. Multivariate linear regression using ADPN concentration at baseline, 3 months and 12 months, respectively, as the dependent variable was performed for further investigation of the dependence of various clinical and laboratory observations on the ADPN concentration before and after Tx. The following independent variables were included in the multivariate regression model: Age, BMI, ISI, eGFR, use of tacrolimus or ciclosporine and accumulated prednisolone dose within the first 90 days. Uni- and multivariate

ordinal logistic regression analyses using the proportional odds ratio model were performed to identify risk factors for deterioration of glucose tolerance at 12 months following Tx including development of NODM. Glucose tolerance at 12 months (grouped categorically into three groups (i) NGT, (ii) IGT and/or IFG and (iii) NODM) was used as the dependent variable and baseline and post-Tx data of ADPN, ISI, vWF, total cholesterol, BMI, mean arterial pressure (MAP), age, ESRD duration, former Tx, use of tacrolimus after Tx and prednisolone dose within the first 90 days were tested as independent variables. The odds ratio refer to the risk of aggravation of the glucose tolerance from one category to another (e.g. from NGT to IGT or from NGT to NODM). A *P*-value <0.05 determines significance.

Results

Demographic and clinical results

The patients from the two groups were well matched according to most clinical and demographic parameters (Table 1). The Tx group was younger and had a shorter duration of ESRD ($P = 0.03$ and $P = 0.001$ respectively). Baseline ADPN concentrations were similar between the Tx group and the uraemic group (P = NS). ADPN levels declined at 3 months in the Tx group ($P < 0.0001$) and this level was maintained at 12 months ($P < 0.0001$). eGFR and BMI increased at both follow-up visits in the Tx group ($P < 0.0005$ and $P < 0.05$ respectively). ISI declined significantly and FPG and HbA1c increased significantly at both visits after transplantation in the Tx group. CRP and uric acid both increased following Tx, however, only uric acid on a significant level ($P = 0.0001$ and $P = 0.01$ at 3 and 12 months respectively). Total plasma cholesterol and triglycerides increased at 3 months in the Tx group ($P < 0.005$), but declined towards baseline levels at 12 months (P = NS). MAP declined after Tx ($P < 0.05$ at 3 and 12 months) despite a lowering of the number of antihypertensives used. All parameters, including ADPN and glucose tolerance, remained unaltered with no significant changes in the uraemic control group between 0 and 12 months (Table 2). Clinical data are summarized in Table 2.

Immunosuppressive treatment

The immunosuppressive treatments in the Tx group at 12 months were as follows: tacrolimus (37%), cyclosporine (56%), prednisolone (91%) and mycophenolate mofetil (96%) (Table 2). Accumulated prednisolone dose 3 months after Tx varied between centres with an individual range from 0 to 6455 mg. The mean daily dose of prednisolone was 13 mg at 3 months and 6 mg at 12 months.

Statistical analyses

ADPN concentrations were significantly (positive) correlated with ISI and HDL-cholesterol and inversely correlated with eGFR, BMI, WHR and triglyceride levels, respectively, at baseline and 12 months following Tx. Correlation between ADPN and ISI, HDL-cholesterol and triglyceride, respectively, were lost at 3 months. No correlation was found between ADPN and total plasma cholesterol, $OGTT_{PG\ 120\ min}$, FPG, CRP, plasma uric acid, MAP or vWF at any time before or after Tx (Table 3). Correlations between ΔeGFR and ΔADPN at 3 and 12 months were insignificant ($P = 0.65$ and $P = 0.94$ respectively). Because of the uncertainty in using eGFR in ESRD patients [31], we tested the correlation using baseline eGFR = 0 ml/min, 5 ml/min, 10 ml/min and 15 ml/min, separately, in the calculation of the delta value, resulting in similar, insignificant results (data not shown). In a variance analysis, the Tx group was divided into two subgroups according to the median ADPN level at baseline (above/below 15 mg/l; Table 4). ADPN was significantly higher and BMI significantly lower before Tx and 3 and 12 month after Tx in the group with ADPN above the median at baseline compared with the group with ADPN below the median. eGFR and ISI were independent of baseline ADPN concentration at all times pre- and post-transplantation (Table 4), which were also the case for vWF, lipids, WHR, FPG, $OGTT_{PG\ 120\ min}$, MAP, CRP and uric acid (data not shown). In the multivariate linear regression analysis, BMI as the only parameter tested was inversely associated with ADPN at baseline ($\beta = -0.87$; $P = 0.003$). At 3 months post-Tx, eGFR and use of tacrolimus or ciclosporine were all significant and inversely independent predictors of the ADPN concentration, however, only eGFR remained significant at 12 months. ADPN levels were independent of ISI at baseline, but significantly associated 12 months after Tx ($\beta = 0.50$; $P = 0.03$). Age and accumulated prednisolone dose were not associated with ADPN at any time pre- or post-Tx ($P > 0.17$) (Table 5).

In the uni- and multivariate ordinal logistic regression model, the Tx group was divided into subgroups according to glucose tolerance at 12 months (NGT, IGT/IFG or NODM). Baseline ADPN concentration did not predict development of NODM following Tx or even deterioration of the glucose tolerance (OR: 0.90–1.14; $P = 0.85$). The univariate analysis showed a significant risk for developing prediabetes or NODM in patients with high age, vWF and/or total cholesterol levels at baseline, while high ISI at baseline protected against deterioration of the glucose tolerance. High BMI at baseline showed a nonsignificant trend towards aggravation of the glucose tolerance. However, only low ISI and high age remained

Table 3. Correlation analysis for the Tx group.

		Plasma ADPN at baseline	Plasma ADPN 3 months after Tx	Plasma ADPN 12 months after Tx
eGFR (ml/min)	*r*-value	−0.31	−0.38	−0.30
	P-value	0.02	0.004	0.03
BMI (kg/m^2)	*r*-value	−0.44	−0.29	−0.27
	P-value	0.0006	0.03	0.045
ISI (index)	*r*-value	0.29	0.23	0.34
	P-value	0.03	0.09	0.01
WHR	*r*-value	−0.27	−0.34	−0.37
	P-value	0.048	0.02	0.0045
Total plasma cholesterol (mmol/l)	*r*-value	0.06	0.17	0.06
	P-value	0.65	0.20	0.65
Plasma HDL-cholesterol (mmol/l)	*r*-value	0.45	0.16	0.45
	P-value	0.0005	0.23	0.0006
Plasma triglyceride (mmol/l)	*r*-value	−0.48	−0.04	−0.23
	P-value	0.0002	0.80	0.09
Plasma CRP (mg/l)	*r*-value	−0.13	−0.20	−0.02
	P-value	0.32	0.14	0.86
Plasma uric acid (mmol/l)	*r*-value	−0.15	0.14	0.08
	P-value	0.24	0.31	0.56

ADPN, adiponectin; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; ISI, insulin sensitivity index; Tx, kidney transplantation; WHR, waist–hip ratio. Data from FPG (fasting plasma glucose), MAP (mean arterial pressure), $OGTT_{PG\ 120\ min}$ (plasma glucose 120 min following oral glucose tolerance test) and vWF (von willebrand factor) were all nonsignificant and are not shown.
Spearman's rank correlation coefficients.

Table 4. Variance analysis for the Tx group. Relation to baseline ADPN concentration.

	Plasma ADPN at baseline	*n*	Baseline	3 months after Tx	12 months after Tx
Plasma ADPN (mg/l)	High (>15 mg/l)	32	22.3 ± 7.0***	12.1 ± 4.6***	11.9 ± 4.2***
	Low (<15 mg/l)	25	9.9 ± 3.0	8.0 ± 3.1	7.9 ± 3.4
eGFR (ml/min)	High (>15 mg/l)	32	<15†	69.1 ± 23.0	73.9 ± 25.2
	Low (<15 mg/l)	25	<15†	79.2 ± 14.7	83.3 ± 18.3
BMI (kg/m^2)	High (>15 mg/l)	32	23.2 ± 3.3*	24.1 ± 3.5**	25.1 ± 4.4*
	Low (<15 mg/l)	25	26.0 ± 4.0	27.0 ± 3.8	27.9 ± 4.5
ISI (index)	High (>15 mg/l)	32	7.5 ± 4.0	5.0 ± 3.3	5.4 ± 3.1
	Low (<15 mg/l)	25	5.9 ± 3.7	4.8 ± 4.7	4.2 ± 2.1

ADPN, adiponectin; BMI, body mass index; eGFR, estimated glomerular filtration rate; ISI, insulin sensitivity index; Tx, kidney transplantation.
Data are presented as mean ± SD and numbers (*n*).
Kruskal–Wallis one-way analysis of variance. Tx patients with high plasma ADPN at baseline vs. low plasma ADPN at baseline: $*P < 0.05$, $**P < 0.005$, $***P < 0.0005$.
†<15 ml/min has been used as an estimate of eGFR in the ESRD patients, as calculation of eGFR is not validated in this group of patients [31].

significant risk factors in the adjusted, multivariate model, while former Tx turned out to confer significant protection against aggravation of the glucose tolerance (Table 6). Eight patients (14%) in the Tx group developed diabetes at 12 months while that was the case in two patients (5%) in the uraemic control group ($P < 0.01$; Table 2). One patient required insulin treatment for a short period while the remaining patients were treated with diet alone. Three patients (21%) among the 14 patients initially receiving tacrolimus-developed NODM compared with 5 (11%) of the 43 patients who initially received cyclosporine; however, the ordinal logistic regression model could not demonstrate a significant difference between the two immunosuppressive regimens. The average accumulated prednisolone dose was 0.5 gram (22%) higher in patients developing NODM or prediabetes compared with patients with normal glucose tolerance after Tx; this difference, however, also did not reach statistical significance in the ordinal logistic regression model (Table 6).

Table 5. Multivariate linear regression for the Tx group.

	Baseline		3 months after Tx		12 months after Tx	
Plasma ADPN	Regression coefficient	*P*-value	Regression coefficient	*P*-value	Regression coefficient	*P*-value
Age (years)	0.03	0.71	−0.02	0.66	0.03	0.64
BMI (kg/m^2)	−0.87	0.003	−0.25	0.09	−0.02	0.91
eGFR (ml/min)	−0.44	0.11	−0.09	0.005	−0.07	0.04
ISI (index)	0.09	0.77	−0.03	0.82	0.50	0.03
Accumulated prednisolone dose within 90 days (g)	–	–	0.35	0.39	0.57	0.17
Use of tacrolimus (Y/N)	–	–	−9.99	0.001	−0.63	0.78
Use of ciclosporine (Y/N)	–	–	−7.86	0.009	−2.12	0.34
R^2	0.25		0.45		0.30	

BMI, body mass index; eGFR, estimated glomerular filtration rate; ISI, insulin sensitivity index; OGTT, oral glucose tolerance test; N, no; Y, yes.
Multivariate linear regression analysis using adiponectin (ADPN) concentration at baseline and 3 and 12 months after Tx as the dependent variable.

Table 6. Risk assessment of baseline and post-Tx parameters determining aggravation of glucose tolerance after Tx.

	No diabetes		NODM	OR (95% Confidence interval)		
12-month OGTT	NGT *n* = 32	Prediabetes *n* = 17	DM *n* = 8	Crude	Adjusted	*P*-value
Baseline and post-Tx data						
Baseline plasma ADPN (mg/l)	16.6 ± 7.6	17.9 ± 10.4	15.6 ± 6.3	0.99 (0.95–1.03)	1.01 (0.90–1.14)	0.85
Baseline ISI (index)	7.9 ± 3.3	5.9 ± 4.9	4.2 ± 1.6	0.82 (0.69–0.97)	0.83 (0.70–0.98)	0.01
Baseline plasma vWF (kIU/l)	1.6 ± 0.7	2.1 ± 0.9	1.8 ± 0.7	1.82 (1.29–2.58)	2.16 (0.78–6.02)	0.14
Baseline total plasma cholesterol (mmol/l)	4.9 ± 1.3	4.6 ± 0.9	6.3 ± 1.6	1.44 (1.20–1.73)	1.12 (0.68–1.86)	0.66
Age at Tx (years)	35 ± 12	45 ± 12	44 ± 12	1.06 (1.02–1.11)	1.03 (1.01–1.11)	0.01
Baseline BMI (kg/m^2)	23.6 ± 3.3	24.9 ± 4.7	26.7 ± 3.4	1.14 (0.99–1.31)	1.02 (0.82–1.29)	0.84
Change in BMI (0–12 months) (kg/m^2)	1.8 ± 2.5	2.3 ± 1.4	1.1 ± 1.7	0.97 (0.76–1.24)	0.78 (0.53–1.14)	0.84
Baseline MAP (mmHg)	103 ± 12	102 ± 14	109 ± 20	1.01 (0.98–1.09)	1.05 (0.99–1.11)	0.07
Former Tx, *n* (%)	8 (25)	3 (18)	0	0.36 (0.08–1.55)	0.05 (0.003–0.88)	0.04
ESRD duration at baseline (months)	7 (0–134)	25 (0–108)	30 (5–48)	1.02 (1.00–1.03)	1.02 (0.98–1.06)	0.30
Accumulated prednisolone dose within 90 days (g)	2.39 ± 1.26	2.93 ± 1.20	2.93 ± 1.65	1.34 (0.91–2.08)	0.58 (0.26–1.30)	0.19
Use of tacrolimus, *n* (%)	7 (22)	4 (24)	3 (37)	1.54 (0.49–4.86)	4.21 (0.50–35.26)	0.19

ADPN, adiponectin; BMI, body mass index; DM, diabetes mellitus; ISI, insulin sensitivity index; MAP, mean arterial pressure; vWF, von willebrand factor.
Data are presented as mean ± SD, median (range), numbers (*n*) and per cent (%). Baseline and post-Tx data are grouped according to glucose tolerance 12 months after Tx in normal glucose tolerance (NGT), prediabetes (impaired glucose tolerance and/or impaired fasting glucose) or new onset diabetes mellitus (NODM). Odds ratio (OR) refers to the risk of aggravation of the glucose tolerance. Crude values indicate results from the univariate ordinal logistic regression model, whereas adjusted values refer to the multivariate ordinal logistic regression model corrected for the remaining parameters. *P*-values refer to the multivariate test. Glucose tolerance was used as dependent variable.

Discussion

The primary findings of the present prospective study were: (i) Pretransplant ADPN concentration did not predict development of NODM or even deterioration of glucose tolerance 12 months following Tx, (ii) Several parameters, including markers of kidney function, cardiovascular risk factors and glucometabolic parameters were correlated with and independent predictors of the ADPN concentration, (iii) Correlation and association between ADPN and markers of IR and CVD differed depending on time interval following Tx, suggesting that ADPN results must be interpreted cautiously within the first months following Tx, and (iv) Tx was accompanied by a significant reduction in ADPN concentration. Our results are in accordance with Cudek *et al.* [20] and Adamczak *et al.* [34], who also demonstrated a significant reduction in plasma ADPN after Tx, whereas Taherimahmoudi *et al.* [21] found unaltered concentrations before and after Tx. We found eGFR and ADPN

concentration to be correlated before and after Tx, whereas ΔeGFR and ΔADPN were uncorrelated following Tx. When adjusting for age, BMI, ISI and use of immunosuppressive agents, eGFR did predict ADPN levels independently at 3 and 12 months after Tx. These findings suggest that the improved kidney function observed following Tx is a contributory cause for the reduced ADPN concentration; however, that factors other than the kidney function are also involved. Factors like IR, inflammation, BMI, endothelial function and markers of CVD have been suggested to affect ADPN levels [1,2,4,5,7–10] and our results support these findings; the decreased ADPN concentration following Tx was accompanied by a significant reduction in ISI and a significant increase in BMI, FPG and HbA1c 12 months after Tx. These associations are supported by the results of the correlation analysis and the multivariate regression analysis at baseline and 12 months following Tx. However, correlation between ADPN and ISI, HDL-cholesterol and triglyceride, respectively, were lost at 3 months. This is most likely explained by the higher doses of immunosuppression at 3 months compared with 12 months, which is supported by the highly significant associations between ADPN and use of ciclosporine or tacrolimus, respectively, at 3 months in the regression analysis. Persistent inflammation did not seem to be involved in the process as CRP was unaltered at 3 and 12 months after Tx and uric acid was increased at both 3 and 12 months following Tx, when compared with baseline data. Furthermore, CRP and uric acid were uncorrelated with ADPN at any time before or after Tx. Taken together, our results indicate that the metabolic and renal state of the patients at 3 months post-Tx is not stable when it comes to interpretation of ADPN concentrations, even despite a normal kidney allograft function has been obtained. Therefore, the difference in our findings at 3 months and 12 months following Tx suggests that ADPN results must be interpreted cautiously within the first months following Tx.

Low ISI, high age and no former Tx were the only significant pretransplant markers for deterioration of the glucose tolerance as previously demonstrated [26], whereas ADPN levels before Tx did not appear to be an independent risk factor for deterioration of glucose tolerance including development of NODM 12 months after Tx in our study. This finding is in conflict with two studies by Bayes *et al.* [23,24]. In one of these, 199 kidney allograft recipients were examined consecutively. 23% developed NODM of whom 77% were diagnosed within 3 months from Tx. The patients who developed NODM had significantly lower pretransplant plasma ADPN levels [24]. In the second study, 68 patients were studied before and after Tx. 31 patients (46%) were diagnosed with NODM at a median of 2 months after Tx and this subgroup had significantly lower pretransplant ADPN levels compared with the Tx patients who did not develop NODM [23]. However, it was previously demonstrated that the prevalence of NODM was highest within the first months following Tx and that glucose tolerance, and consequently the prevalence of NODM, subsequently improved [35]. This is in accordance with our data at 3 and 12 months and might explain the discrepancy between the findings in our study and the two from Bayes *et al.*

As indicated, results are conflicting and the role and potentially predictive character of ADPN in patients with ESRD and following successful Tx remains to be fully understood. The question whether ADPN levels are a cause or a consequence of the many changes appearing following Tx (i.e. improvement of kidney function, daily intake of diabetogenic immunosuppressive agents, weight gain, and changes in lipids and blood pressure) also remain to be fully elucidated. Larger prospective studies, including subgroups of patients with different stages of CVD, glucose tolerance, BMI and inflammation, should be initiated to achieve this information. In our study, all parameters, including ADPN and glucose tolerance, remained unaltered with no significant changes in the uraemic control group between 0 and 12 months, thus excluding the time factor separately as a possible confounder. Our study has some limitations. The Tx group is heterogeneous and contains patients receiving different immunosuppression regimens and patients with and without previous Tx's. These issues might affect the glucose tolerance and/or ADPN levels. The method used for analysing ADPN includes several isoforms. The separate isoforms have been given individual properties and the high molecule isoform has been shown to correlate best with insulin sensitivity [36], and ideally we should have analysed and investigated the isoforms individually.

In conclusion, we found ADPN concentrations to decline significantly following successful Tx. Pretransplant ADPN concentration did not predict development of NODM or even deterioration of the glucose tolerance 12 months following Tx. Our results suggest that ADPN results must be interpreted cautiously within the first months following Tx and that ADPN concentrations are determined and influenced by multiple factors including kidney function, IR, BMI, endothelial function, risk factors of CVD, use of immunosuppressive agents and maybe other unknown factors.

Authorship

TI: participated in research design and performance of the study, data analysis and writing of the paper. MH: participated in research design and performance of the

study, in data analysis and writing of the paper. MB: participated in data analysis and writing of the paper. KAJ: participated in research performance, data analysis and writing of the paper. FTN: participated in research performance, data analysis and writing of the paper. JMH: participated in research performance, data analysis and writing of the paper. AF: participated in research design, data analysis and writing of the paper. BFR: participated in research design, data analysis and writing of the paper.

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