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ORIGINAL ARTICLE

Adipokines in type 1 diabetes after successful pancreas transplantation: normal visfatin and retinol-binding-protein-4, but increased total adiponectin fasting concentrations

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Summary

Objective In type 1 diabetes mellitus (T1DM), the release of many hormones, not only from beta-cells, but also from adipocytes (adipokines) may be altered. After successful pancreas–kidney-transplantation (PKTx), T1DM patients can revert to a nondiabetic metabolism, but it is unclear whether alterations of adipokines are still present after PKTx.

Design, patients and measurements Concentrations of adipokines [visfatin, retinol-binding protein-4 (RBP-4), adiponectin, high molecular weight (HMW) adiponectin] were measured at fasting in 10 PKTx and in 19 T1DM. Nondiabetic healthy controls (CON, $n = 9$) and six nondiabetic patients after kidney transplantation (KTx) were examined as control groups. In PKTx, KTx and CON, indices of insulin sensitivity (OGIS) and beta cell function (adaptation index, AI) were calculated from 75 g oral glucose tolerance test (OGTT) data.

Results Fasting serum visfatin (T1DM: 56 ± 4 μg/l, PKTx: 42 ± 6 μg/l, KTx: 39 ± 3 μg/l, CON: 40 ± 3 μg/l) and RBP-4 (T1DM: 490 ± 26 μg/l, PKTx: 346 ± 39 μg/l, KTx: 401 ± 13 μg/l, CON: 359 ± 36 μg/l) was increased by 40% and 36%, respectively (each $P < 0{\cdot}03$) in T1DM only. Levels were positively correlated with HbA1c in all subjects (visfatin: $r = 0{\cdot}43$, $P < 0{\cdot}004$; RBP-4: $r = 0{\cdot}46$, $P < 0{\cdot}03$). Fasting plasma adiponectin was 80% higher in T1DM and in PKTx (T1DM: 18 ± 2 mg/l, PKTx: 18 ± 3 mg/l, KTx: 12 ± 3 mg/l, CON: 10 ± 1 mg/l; $P < 0{\cdot}04$) and was positively correlated with diabetes duration ($r = 0{\cdot}37$, $P < 0{\cdot}02$). HMW/total adiponectin ratio was increased in T1DM ($P < 0{\cdot}02$). PKTx displayed a normoglycaemic metabolism as insulin sensitive as CON, but AI was lower than in CON and KT ($P < 0{\cdot}01$).

Conclusions T1DM after successful PKTx show normal fasting visfatin and RBP-4 levels and HMW-adiponectin/adiponectin-ratio, which are elevated in T1DM, whereas total adiponectin levels are similarly increased in T1DM and PKTx patients.

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Introduction

In type 1 diabetes mellitus (T1DM), evidence has been provided for altered release of hormones from adipocytes (adipokines).[1,2] This is regarded as another pathophysiological feature in addition to the abnormal regulation of glucose and lipid metabolism resulting from the lack of endogenous insulin and C-peptide secretion in T1DM.[3]

The release of the adipokine visfatin, which is predominantly produced and secreted by visceral adipose tissue and macrophages, is regulated by circulating glucose and insulin concentrations. Visfatin was suggested to have insulin-mimetic effects via direct binding to and activation of the insulin receptor.[4] However, more recent data indicate that visfatin directly stimulates insulin secretion.[5] Plasma visfatin concentrations were reported to be increased in patients with type 1 and type 2 diabetes[1,6–8] and to be negatively related to beta cell function.[1] The recently discovered adipokine retinol-binding protein-4 (RBP-4) was suggested to impair whole body insulin sensitivity.[9] In contrast, our group did not find any association of increased serum RBP-4 levels with insulin resistance in nondiabetic humans.[10] However, to the best of our knowledge, no reports on RBP-4 in T1DM patients or animal models have yet

been published. The adipokine adiponectin mainly acts on skeletal muscle and liver, possibly by increasing insulin sensitivity.[11] Circulating levels of adiponectin are reduced in obesity and type 2 diabetes.[6] In T1DM patients, total adiponectin concentrations are increased,[12] depending on T1DM duration[2] and the presence of diabetic microvascular complications.[12] In addition, the relative fraction of the high molecular weight (HMW) adiponectin isoform is up-regulated in T1DM patients.[13]

In T1DM with end stage renal disease, combined kidney pancreas transplantation (PKTx) is currently the treatment of choice. Successful PKTx results in sustained normoglycaemia without the necessity of exogenous insulin supply and protects the body from progression of long-term diabetic complications and the effects of glucolipotoxicity.[14,15] We hypothesized that in T1DM after PKTx long-term normoglycaemic metabolism combined with restored endogenous insulin and C-peptide secretion could at least in part reverse some of the alterations of adipokines seen in T1DM.

To this aim, we assessed the concentrations of visfatin, total adiponectin, HMW-adiponectin and RBP-4 in type 1 diabetic patients after PKTx, in type 1 diabetic patients (T1DM), in healthy controls (CON) and, in order to avoid biases because of immunosuppressive agents, also in nondiabetic patients after kidney transplantation (KTx). In PKTx, CON and KT subjects, we also assessed parameters of beta cell function and insulin sensitivity to verify if the previously found minor defects of beta cell function in type 1 after PKTx,[14] could also influence the secretion of adipokines.

Research design and methods

Participants

Ten T1DM patients after successful combined pancreas and kidney transplantation (PKTx), nineteen type 1 diabetic patients (T1DM), nine nondiabetic controls (CON) and six nondiabetic patients after successful kidney transplantation (KTx) were examined. The groups did not differ in age, gender and BMI (Table 1). PKTx patients had received a whole pancreas graft with systemic venous anastomosis to the iliac vein[14] 4·3 ± 0·5 years prior to the study and had a comparable duration of type 1 diabetes as the T1DM group. At the time of examination, the immunosuppressive regimen in PKTx, who were free of glucocorticoids for at least 5 months, included tacrolimus (n = 9) or sirolimus (n = 1) combined with either mycophenolate mofetil (n = 7) or azathioprine (n = 3). KTx underwent successful kidney transplantation 11·0 ± 1·5 years prior to the study. At the time of examination, the immunosuppressive regimen in KTx included prednisolone (n = 5) and tacrolimus (n = 1) combined with either mycophenolate mofetil (n = 4) or azathioprine (50–75 mg/day, n = 2) or

Table 1. Anthropometrical and laboratory characteristics, as well as dynamic areas under the curve (ΔAUC) of glucose, insulin, C-peptide, insulin sensitivity (OGIS) and beta cell function (adaptation index) during the OGTT in type 1 diabetic patients after pancreas and kidney transplantation (PKTx), nondiabetic humans after kidney transplantation (KTx), type 1 diabetic patients (T1DM) and nondiabetic controls (CON)

	PKTx	KTx	T1DM	CON
n (f/m)	10 (4/6)	6 (3/3)	19 (8/11)	9 (4/5)
Age (years)	47 ± 3	50 ± 5	49 ± 1	47 ± 3
Time after diabetes onset (years)	33 ± 2	–	29 ± 3	–
Transplant duration (years)	4 ± 0[&]	11 ± 3	–	–
BMI (kg/m^2)	25 ± 1	25 ± 1	26 ± 1	24 ± 1
SBP/DBP (mmHg)	154 ± 6 [$,‡]/85 ± 4	146 ± 12 /82 ± 6	133 ± 3/80 ± 2	127 ± 6/77 ± 3
HbA1c (%)	5·5 ± 0·1	5·4 ± 0·2	7·4 ± 0·3[*,&,§]	5·4 ± 0·1
Creatinine (μmol/l)	134 ± 11[§,‡]	135 ± 5[§,‡]	88 ± 3	75 ± 2
Glomerular filtration rate (ml/min/1·73m^2)	49·8 ± 4·3[§,‡]	45·8 ± 4·6[§,‡]	77·3 ± 3·1	89·6 ± 3·9
Triglycerides (mmol/l)	1·02 ± 0·16	1·43 ± 0·18	0·93 ± 0·16	0·95 ± 0·11
Total cholesterol (mmol/l)	4·45 ± 0·21[+,$]	5·66 ± 0·65	5·35 ± 0·18	5·09 ± 10·26
HDL cholesterol (mmol/l)	1·84 ± 0·16	1·94 ± 0·39	2·12 ± 0·13	2·07 ± 0·10
LDL cholesterol (mmol/l)	2·43 ± 0·13[+,$]	3·28 ± 0·26	3·10 ± 0·16	2·87 ± 0·31
Fasting plasma glucose (mmol/l)	5·7 ± 0·2	5·4 ± 0·2	10·2 ± 1·1[*,&,§]	5·2 ± 0·1
Oral glucose tolerance test				
Plasma glucose 120 min (mmol/l)	7·6 ± 0·6 [$]	7·2 ± 0·8	–	5·2 ± 0·2
Fasting plasma insulin (pmol/l)	140 ± 12 [$,§]	91 ± 17	–	71 ± 7
Fasting plasma C-peptide (pmol/l)	803 ± 58	1199 ± 259[§,¶]	–	559 ± 85
ΔAUC Glucose (mmol l^{-1}·min)	326 ± 50	328 ± 81	–	203 ± 53
ΔAUC Insulin (nmol/l·min)	38 ± 4	72 ± 21[¶]	–	40 ± 8
ΔAUC C-Peptide (nmol/l·min)	161 ± 14	318 ± 60[*]	–	224 ± 19
OGIS (ml·min^{-1}·m^{-2})	362 ± 13	333 ± 34[#]	–	411 ± 17
Adaptation index (nmol·min^{-1}·m^{-2})	58 ± 5[#,$]	96 ± 8	–	91 ± 7

ΔAUC, dynamic area under the curve; BMI, body mass index; HDL, high density lipoprotein cholesterol; LDL, low density lipoprotein cholesterol; OGIS, oral glucose insulin sensitivity index; SBP/DBP, systolic and diastolic blood pressure;
#$P < 0·05$ *vs.* CON; +$P < 0·05$ *vs.* T1DM; $$P < 0·05$ *vs.* KTx; ¶$P < 0·05$ *vs.* PKTx.
§$P < 0·01$ *vs.* CON; ‡$P < 0·01$ *vs.* T1DM; &$P < 0·01$ *vs.* KTx; *$P < 0·01$ vs. PKTx.
Data are means ± SEM. ANOVA with Bonferroni *post hoc* test.

cyclosporin A ($n = 4$). In PKTx, KTx and CON, normal fasting plasma glucose, glycated haemoglobin A1c (HbA1c) <6·5% and stable serum creatinine were required for participation in the study. None of the PKTx, KTx and CON was using insulin or any other hypoglycaemic agent, whereas T1DM were on insulin treatment. The presence of micro and macrovascular complications and neuropathy was assessed during the annual routine examinations of T1DM and PKTx patients at our outpatient ward.

All participants gave their written informed consent to the protocol, which had been approved by the Local Ethics Committee.

Blood sampling

Blood sampling from an antecubital vein was performed after a 12-h overnight fast in T1DM. Blood was rapidly centrifuged. Serum and plasma aliquots were stored at −70 °C until further analysis.

Oral glucose tolerance test

In PKT, KT and CON a 75 g oral glucose tolerance test (OGTT) was performed after a minimum 12-h overnight fast. A catheter (Venflon®, Becton–Dickinson, Sweden) was inserted into an antecubital vein of the forearm for blood sampling. After collection of basal samples, participants were given the standard dose of 75 g glucose in H_2O solution (Glukodrink®, Roche Diagnostics, Vienna, Austria) within 5 min, and were maintained on bed rest. Venous blood samples for measurement of metabolites and hormones were drawn in the fasting state and at 10, 20, 30, 40, 60, 90, 120, 150 and 180 min after glucose load. Blood was rapidly centrifuged, and serum/plasma aliquots were stored at −70 °C until further analysis.

Laboratory measurements

HbA1c and serum concentrations of triglycerides, total, LDL-, and HDL-cholesterol and creatinine were measured using routine laboratory methods. Plasma concentrations of glucose, insulin and C-peptide were measured as previously described.[14] Fasting concentrations of serum visfatin and RBP-4 and plasma adiponectin were analysed using commercially available ELISA kits (visfatin, RBP-4, HMW-adiponectin) and RIA kits (total adiponectin: Linco Research Inc., St. Charles, MO, USA) with interassay and intraassay coefficients of variation <10%. All samples from the study were measured within the same assay, except for the assessment of HMW adiponectin.

Calculations of insulin sensitivity and beta cell function

Insulin sensitivity was assessed with the oral glucose insulin sensitivity index (OGIS), which represents glucose clearance taking into account known relationships between glucose disappearance and insulin.[16] OGIS has been extensively validated against the gold standard euglycaemic hyperinsulinaemic clamp and already widely applied in human studies.[16] In PKTx, insulin and C-peptide are delivered directly into the systemic circulation, whereas in CON and KTx they undergo first pass through the liver. As the liver does not play a significant role in C-peptide clearance, we compared beta cell dynamics between the two groups by analysing the kinetics of C-peptide.[17,18] ΔAUC were calculated by subtracting from the total AUC, calculated with the trapezoidal rule, the basal AUC, i.e. the fasting concentration × 120 min. As beta cell function cannot be evaluated unless related to ambient insulin sensitivity (42), we have calculated the product between insulin sensitivity and the beta cell function index. This product, sometimes called adaptation index ($AI = OGIS \times \Delta AUC_{C\text{-peptide}}$; $nmol \cdot min^{-1}\ m^{-2}$) was previously introduced for the intravenous glucose test (43) but it has already been used in the OGTT to estimate the ability of the beta cell to increase its own response to compensate for the increased insulin resistance (43). Glomerular filtration rate was calculated using the modification of diet in renal disease 2 (MDRD) formula.[19] HMW/total-adiponectin-ratios were calculated by dividing HMW-adiponectin by total adiponectin fasting plasma concentrations.[13]

Statistics

Differences between groups were assessed by performing χ^2 tests for categorical variables. Before further analysis, the distribution of the variables was tested by applying the Kolmogorov-Smirnov test, which showed that all of the continuous variables were normally distributed. Continuous variables were analysed with ANOVA following Bonferroni or LSD *post hoc* test (as indicated). Linear correlations were based on Pearson's product-moment correlations. Statistical analyses were performed using SPSS® (SPSS Inc., Chicago, IL, USA). Data are given as means ± SEM. Differences between groups at $P \leq 0{\cdot}05$ were considered statistically significant.

Results

Participants' characteristics (Table 1)

The four groups did not differ in age, gender and BMI. PKTx and T1DM had comparable diabetes disease duration. T1DM had a higher HbA1c and higher fasting plasma glucose levels than the other three groups ($P < 0{\cdot}01$). Both PKTx and KTx had equally increased serum creatinine ($P < 0{\cdot}00001$) and decreased glomerular filtration rates ($P < 0{\cdot}00001$) when compared with T1DM and CON. PKTx had lower total and LDL cholesterol in serum ($P < 0{\cdot}05$) than KTx and T1DM, but did not differ in fasting HDL cholesterol and triglycerides. Systolic blood pressure was increased in PKTx ($P < 0{\cdot}01$ *vs* CON and T1DM). Nine patients in the T1DM group and eight in the PKTx group had diabetic retinopathy. Neuropathy was present in all PKTx and in seven T1DM patients. In the PKTx group, two patients had a history of coronary heart disease, three of peripheral arterial occlusive disease and one of cerebrovascular disease. None of the T1DM patients had cerebrovascular, cardiovascular or peripheral vascular disease or microalbuminuria.

Visfatin, total adiponectin, HMW-adiponectin, RBP-4

Visfatin fasting serum concentrations were 40% higher in T1DM, when compared to CON, PKTx and KTx (Fig. 1a;

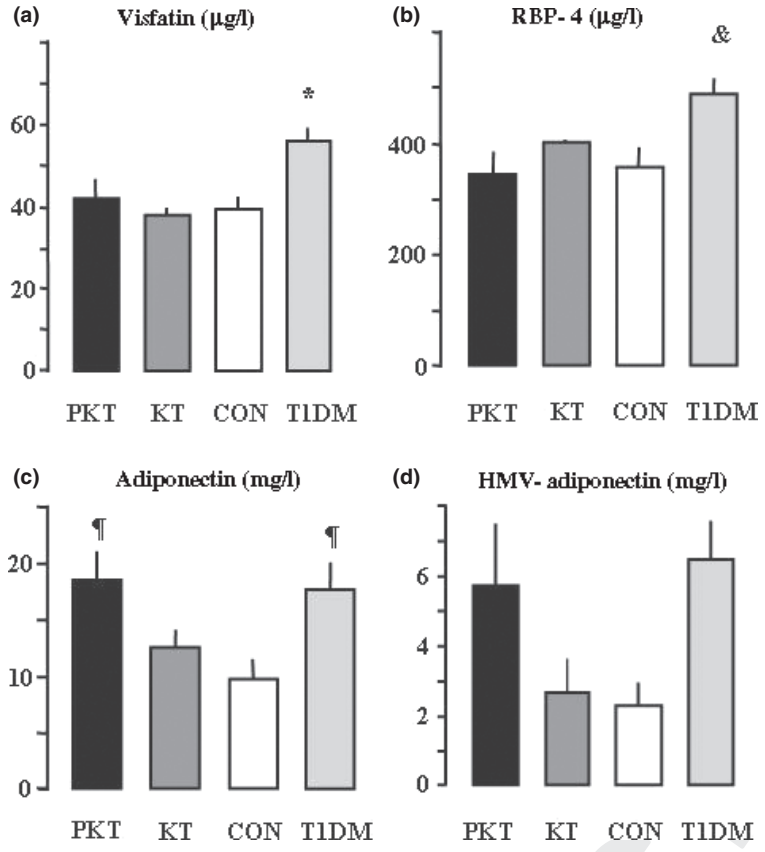

Fig. 1 Fasting concentrations of serum visfatin (a), serum retinol-binding protein-4 plasma adiponectin (c), plasma high molecular weight adiponectin (d) in type 1 diabetic patients after pancreas and kidney transplantation (PKTx, black), nondiabetic kidney transplanted patients (KTx, dark grey), type 1 diabetic patients (T1DM, light grey) and in nondiabetic controls (CON, white). Retinol binding protein 4 (RBP-4), high molecular weight adiponectin (HMW-adiponectin). $^{*}P < 0{\cdot}03$ *vs.* CON, PKTx and KTx; $^{¶}P < 0{\cdot}04$ *vs.* CON and KT; $^{\&}P < 0{\cdot}005$ *vs.* CON, PKTx and KTx. Data are presented as mean ± SEM. ANOVA with LSD *post hoc* test.

$P < 0{\cdot}03$). RBP-4 serum levels were 36% higher in T1DM when compared with CON, PKTx and KTx (Fig. 1b; $P < 0{\cdot}005$). Total-adiponectin plasma concentrations were increased by 80% in both T1DM and PKTx when compared with CON and KTx (Fig. 1c; $P < 0{\cdot}04$). HMW-adiponectin plasma concentrations did not significantly differ in the four groups (Fig. 1d, ANOVA: $P = 0{\cdot}07$). HMW/total-adiponectin ratios were increased in T1DM when compared with KTx and CON (T1DM: 0·34 ± 0·02; KTx: 0·20 ± 0·04; CON: 0·21 ± 0·04; $P < 0{\cdot}02$) and tended to be higher in T1DM than in PKTx (PKTx: 0·26 ± 0·05; $P < 0{\cdot}1$ *vs.* T1DM).

Oral glucose tolerance test, OGIS, adaptation index (Table 1)

Fasting plasma glucose and $\Delta AUC_{glucose}$ were not different in PKTx, KTx and CON, but plasma glucose at 120 min in the OGTT was approximately 45% higher in PKTx than in CON ($P < 0{\cdot}01$). Fasting plasma insulin was approximately twofold higher in PKTx than in CON and in KTx (Table 1, $P < 0{\cdot}05$) and fasting plasma C-peptide was approximately twofold increased in KTx vs. CON and approximately 44% higher than in PKTx (Table 1, $P < 0{\cdot}05$). KTx displayed approximately twofold increased $\Delta AUC_{C\text{-}peptide}$ ($P < 0{\cdot}01$) and $\Delta AUC_{insulin}$ ($P < 0{\cdot}05$) when compared with PKT. AI was reduced by 36% in PKTx when compared with CON and KTx ($P < 0{\cdot}01$). OGIS was decreased in KTx ($P < 0{\cdot}05$) and did not different between PKTx and CON.

Correlation analyses

The correlations of fasting levels of the adipokines visfatin, RBP-4, total adiponectin, HMW adiponectin as well as HMW/total adiponectin ratio with anthropometrical characteristics and routine laboratory measurements in all participants ($n = 44$) and with data obtained from OGTT performed in PKTx, KTx and CON ($n = 25$) are shown in Table 2.

Discussion

The main new findings of this study are that fasting concentrations of visfatin and RBP-4 are increased in T1DM and are normalized in type 1 diabetic patients after successful PKTx to levels comparable with nondiabetic controls, whereas total adiponectin serum concentrations are similarly elevated in PKTx as in T1DM.

Adipokines

In this study, T1DM showed increased fasting concentrations of the adipokines visfatin and adiponectin and HMW/total adiponectin ratio, which is in line with previous studies which demonstrated increased visfatin[1,7] and adiponectin[2,12,13] concentrations in type 1 diabetic patients. Successful PKTx results in long-term normalization of glucose metabolism by substituting all islet cell hormones, as we have shown previously.[14] In the

Table 2 Pearson's product moment correlations of fasting levels of the adipokines visfatin, retinol-binding-protein-4 (RBP-4), total adiponectin, high molecular weight (HMW) adiponectin and HMW/total adiponectin ratio with anthropometrical characteristics, routine laboratory measurements including circulating lipids, renal function and HbA1c in all participants ($n = 44$) and with data obtained from oral glucose tolerance test (OGTT) performed in 25 participants

Overall correlation analyses in all participants ($n = 44$)	Visfatin	RBP-4	Total adiponectin	HMW-adiponectin	HMW/total adiponectin ratio
Age (years)	0·03	0·21	0·10	0·11	0·15
BMI (kg/m^2)	0·12	0·09	−0·19	−0·25	−0·12
Time after diabetes onset	0·29	0·15	0·35*	0·34	0·36*
HbA1c	0·34*	0·34*	0·24	0·27	0·34*
Fasting plasma glucose 0 min (mmol/l)	0·38*	0·41+	0·10	0·16	0·29
Glomerular filtration rate (ml/min/1·73m^2)	0·04	0·00	−0·07	0·04	0·16
Creatinine (μmol/l)	−0·11	−0·10	−0·14	−0·30	−0·41+
Triglycerides (mmol/l)	−0·24	0·09	−0·31	−0·31	−0·32
Total cholesterol (mmol/l)	−0·12	0·20	0·16	0·19	0·20
HDL cholesterol (mmol/l)	0·10	−0·07	0·66#	0·65#	0·55#
LDL cholesterol (mmol/l)	−0·20	0·32*	−0·25	−0·17	−0·07
Visfatin (μg/l)	–	0·20	−0·04	0·20	0·14 *
Total adiponectin (mg/l)	0·24	−0·14	–	0·91#	0·63#
RBP-4 (μg/l)	0·19	–	−0·14	−0·10	0·10
HMW adiponectin	0·20	−0·10	0·91#	–	0·17+
HMW/total adiponectin ratio	0·29	0·10	0·63#	0·84#	–
Correlation analyses in groups with OGTT data (PKTx, KTx and CON; $n = 25$)					
OGTT plasma glucose 120 min (mmol/l)	0·21	0·05	0·46*	0·30	0·10
Fasting plasma insulin (nmol/l)	−0·06	0·05	0·33	0·18	−0·06
Fasting plasma C-peptide (nmol/l)	−0·12	−0·01	0·10	−0·04	−0·12
ΔAUC Glucose (mmol/l·min)	0·24	−0·11	0·40*	0·35	0·23
ΔAUC Insulin (nmol/l·min)	0·16	0·05	0·12	0·14	0·16
ΔAUC C-Peptide (nmol/l·min)	0·22	0·08	0·04	0·12	0·22
OGIS (ml·min^{-1} m^{-2})	0·00	0·08	−0·15	−0·10	0·00
Adaptation index (nmol·min^{-1} m^{-2})	0·32	0·17	−0·05	0·11	0·32

ΔAUC, dynamic area under the curve; BMI, body mass index; CON, control group; HDL, high density lipoprotein cholesterol; KTx, kidney transplanted patients; LDL, low density lipoprotein cholesterol; OGIS, oral glucose insulin sensitivity index; PKTx, pancreas kidney transplanted patients; SBP/DBP, systolic and diastolic blood pressure;
#$P < 0·00001$; +$P < 0·001$; *$P < 0·05$.

present study, in PKTx fasting adiponectin was similarly increased as in the T1DM, whereas fasting visfatin and RBP-4 levels and the HMW/total adiponectin-ratio were comparable to those of CON.

In this study, the novel adipokines visfatin and RBP-4 were, for the first time, examined in type 1 diabetes after successful pancreas transplantation, a condition in which the long-term exposure to glucotoxicity is reversed to a nondiabetic metabolism. Visfatin and RBP-4 serum concentrations were increased only in T1DM, but not in PKTx, who had comparable levels with those in age- and BMI-matched nondiabetic CON. As visfatin concentrations were described as being higher in patients with impaired renal function, we have also examined nondiabetic kidney transplant recipients (KTx) matched with PKTx for serum creatinine. In spite of the reduced renal function in KT and PKT, visfatin and RBP-4 serum concentrations were not different to those in CON. In our study, both visfatin and RBP-4 were positively correlated to HbA1c, but not to diabetes duration.

Visfatin

Visfatin has been suggested to exert dose-dependent insulin-mimetic effects by stimulating glucose transport into muscle and adipocytes and by inhibiting endogenous glucose production.[4] A more recent study in a mouse model showed visfatin to directly stimulate insulin secretion from beta cells.[5] Haider *et al.*[4] demonstrated acute regulation of visfatin by glucose and insulin in healthy subjects and in isolated adipocytes. Elevated visfatin in T1DM and the reversal to normal levels in PKTx suggest an up-regulation of visfatin secretion by adipocytes in response to the chronic hyperglycaemia and hypoinsulinaemia of type 1 diabetes, with subsequent down-regulation after restoration of normoglycaemia and insulin secretion after pancreas transplantation. Thus, the feedback mechanism between recent glycaemia and/or insulinaemia and visfatin release could be confirmed by our study. Since in PKTx, C-peptide and amylin release are restored as well,[14] it cannot be ruled out that these hormones may also contribute to visfatin regulation.

Reports on the recently discovered adipokine RBP-4 are conflicting, as very little is known on the regulation of RBP-4 secretion: One report showed an association between higher RBP-4 levels and impaired whole body insulin sensitivity with decreased glucose transporter-4 expression in adipose tissue.[9,20] On the contrary, a recent study of ours found no correlation between insulin sensitivity and RBP-4 levels in healthy humans.[10] The present study indicates that the concomitant presence of hyperglycaemia and hypoinsulinaemia in T1DM could lead to an up-regulation of RBP-4 secretion, whereas restoration of insulin secretion and normoglycaemia after successful PKTx could reduce RBP-4 to levels of CON again. Thus, it could be suggested that glucose and insulin seem to play a role also in RBP-4 regulation. However, further studies will be needed to elucidate the regulation and role of RBP-4 in glucose metabolism.

In this study, fasting plasma levels of total adiponectin were positively related to diabetes duration, but not to HbA1c. Therefore, our findings seem in line with previous studies which also found increased adiponectin concentrations in type 1 diabetes, which were related to long diabetes duration.[2] Interestingly, the HMW/total adiponectin ratio was increased only in T1DM, but not in PKTx patients. This is in line with a previous study demonstrating increased relative HMW-adiponectin concentrations in T1DM patients.[13] In our study, HMW/total adiponectin ratio was related to diabetes duration, but in contrast to total adiponectin, they were also related to recent glycaemia (HbA1c). HMW-adiponectin concentrations were not different in the four groups, which could be due to limited number of samples and/or the large standard deviation of HMW-adiponectin values.

Total adiponectin was increased not only in T1DM but also in PKTx. These two groups had approximately 30 years long diabetes duration, but PKTx had had a normoglycaemic metabolism for approximately 4 years before the examination. In spite of restoration of insulin-secretion and normoglycaemia, total adiponectin levels are increased in PKTx. Of note, PKTx has been exposed to uraemia and had a high prevalence of microvascular late complications, which could have influenced adiponectin levels. In addition, it cannot be excluded that hyperinsulinaemia in PKTx and insulin treatment in T1DM patients could have contributed to elevated total adiponectin levels, as *in vitro* experiments showed that insulin stimulates adiponectin secretion from adipocytes.[21] Thus, the effects of long-standing exposure to hyperglycaemia, inadequate insulinaemia and uraemia on total adiponectin secretion seem to prevail over years of normoglycaemia. This suggests that adiponectin secretion in T1DM could be influenced by long-term effects of hyperglycaemia, uraemia and microangiopathy rather than by recent glycaemia.

Associations with beta cell function and insulin sensitivity

We previously demonstrated minimal defects of beta cell function in PKTx.[14] According to recent studies, increased visfatin could be related to beta cell dysfunction[1] and increased RBP-4 could be associated with reduced beta cell function in obese and type 2 diabetic patients.[22] In PKTx, KTx and CON, we calculated the AI as parameter of beta cell function from OGTT. AI, which reflects the ability of the beta cells to adapt to the ambient insulin sensitivity, was worse in PKTx than in KTx and CON. Neither AI, nor the ΔAUCs of insulin and C-peptide were correlated with visfatin, or with RBP-4 concentrations.

Insulin sensitivity in PKTx patients was similar to that of nondiabetic controls, whereas KTx subjects were more insulin resistant than CON, likely due to glucocorticoids in their immunosuppressive treatment combination. In line with other studies, we failed to demonstrate an association of visfatin with insulin sensitivity.[6] Also RBP-4 was not related to insulin sensitivity, in agreement with Promitzer *et al.*[10] but in contrast with other reports.[9,20] Although several studies observed a significant positive association between plasma adiponectin and insulin sensitivity in nondiabetic subjects after adjustment for BMI,[23] we could not find associations between plasma total and HMW adiponectin and insulin sensitivity.

Limitations

Some limitations of this study need to be considered. The cross-sectional study design renders no intra-individual comparison pre and post-transplantation. It cannot be ruled out that the different immunosuppressive regimens in PKTx and KTx and the insulin treatment in T1DM could have influenced the hormones measured. Further *in vivo* and *in vitro* studies will be needed to speculate how, and to which extent, the complex and not yet thoroughly elucidated interrelationships among adipokines and islet cell hormones contribute to the reduction of serum visfatin and RBP-4 in T1DM and PKTx.

Conclusions

Type-1 diabetic patients display increased fasting concentrations of the adipokines visfatin, RBP-4 and adiponectin. In type-1 diabetic patients after successful pancreas and kidney transplantation, fasting serum visfatin and RBP-4, which are positively related to glycaemic control, are normalized to the levels of nondiabetic controls, whereas fasting plasma adiponectin, which is positively related to diabetes duration, is increased. Thus, higher visfatin and RBP-4 fasting serum levels in T1DM patients are normal in PKTx in the presence of recent normoglycaemia, whereas plasma adiponectin levels are increased after PKTx.

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Competing interests/financial disclosure

Nothing to declare.

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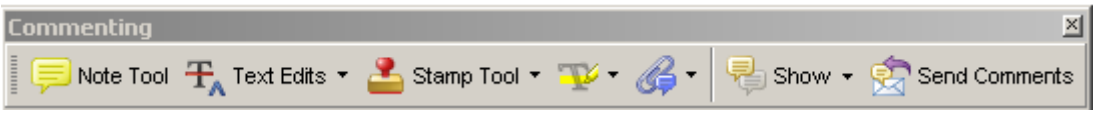

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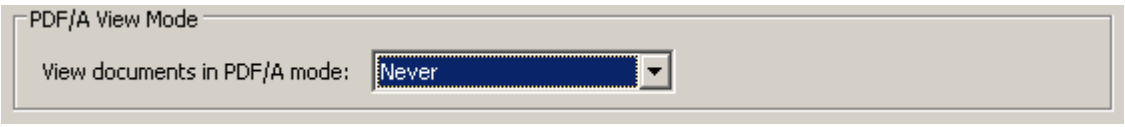

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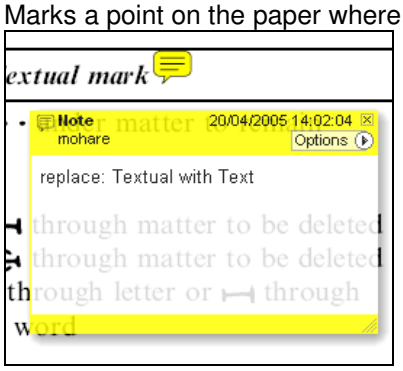

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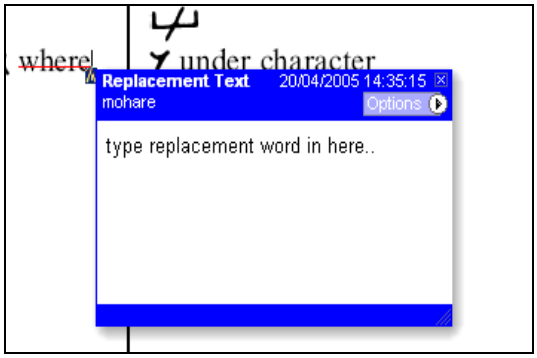

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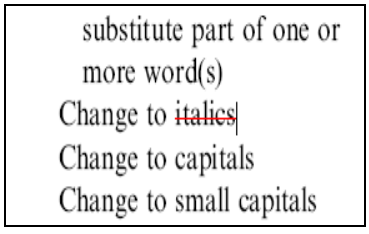

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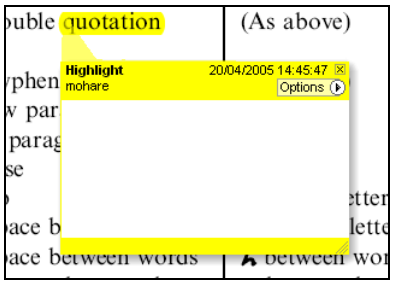

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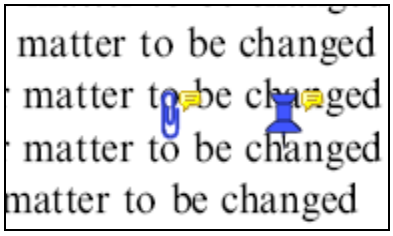

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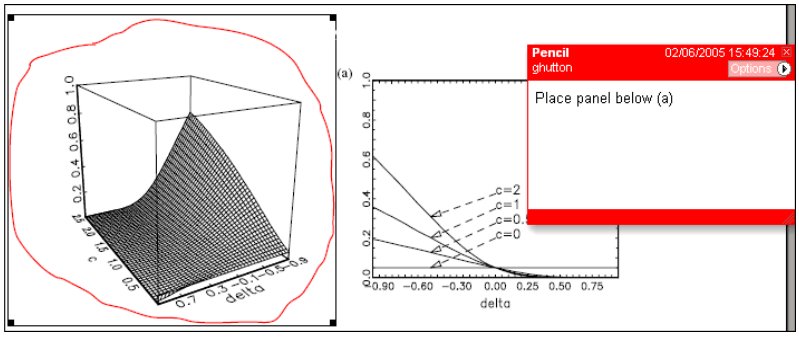

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