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Effects of restoring normoglycemia in type 1 diabetes on inflammatory profile and renal extracellular matrix structure after simultaneous pancreas and kidney transplantation

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Abstract

Aims—Patients with type 1 diabetes and end-stage renal disease with simultaneous pancreas and kidney (SPK) or kidney transplants alone (KA) were recruited 9–12 years post transplantation. We investigated differences between these groups with regard to inflammatory parameters and long-term structural changes in kidneys.

Methods—Blood samples were analyzed by ELISA and multiplex for chemokines, cytokines, growth factors, cell adhesion molecules and matrix metalloproteinases. Kidney graft biopsies were analyzed by electron microscopy for glomerular basement membrane thickness. Heparan- and chondroitin sulfate disaccharide structures were determined by size exclusion chromatography mass-spectrometry.

Results—The SPK and the KA group had average glycated hemoglobin A1c (HbA1c) of 5.8% (40 mmol/mol) and 8.6% (70 mmol/mol) respectively. SPK recipients also had 16.2% lower body mass index (BMI) and 46.4% lower triglyceride levels compared with KA recipients, compatible with an improved metabolic profile in the SPK group. Plasminogen activator inhibitor (PAI-1), C-reactive protein (CRP) and vascular endothelial growth factor (VEGF) were lower in the SPK group. In kidney graft biopsies of the KA-patients an 81.2% increase in average glomerular

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Conflict of interest statement

The authors declare there is no conflict of interest.

basement membrane thickness was observed, accompanied by alterations in heparan sulfate proteoglycan structure. In addition to a decrease in 6-*O*-sulfated disaccharides, an increase in non-*N*-sulfated disaccharides with a corresponding slight decrease in *N*-sulfation was found in kidney biopsies from hyperglycemic patients.

Conclusions—Patients with end stage renal disease subjected to KA transplantation showed impaired inflammatory profile, increased thickness of basement membranes and distinct changes in heparan sulfate structures compared with SPK recipients.

Keywords

Diabetes; Heparan sulfate; Hyperglycemia; Kidney; Proteoglycans

1. Background

Patients with type 1 diabetes have increased risk of macrovascular complications such as atherosclerotic cardiovascular diseases including myocardial dysfunction and stroke [1,2], as well as microvascular complications including neuropathy, retinopathy and nephropathy [3]. Diabetic nephropathy is characterized by hypertension, albuminuria, reduced glomerular filtration rate (GFR) and kidney morphological changes [4]. These overt effects are the long-term consequences of changes in several important signaling systems affected by hyperglycemia [5], by changes in the renin-angiotensin system, increased transforming growth factor β (TGF- β)-activity, promoting fibrotic processes and the activation of immune cells in the circulation and changes in inflammatory pathways, all contributing to systemic endothelial dysfunction [6].

Long-term complications in the kidneys of patients with type 1 diabetes result from a series of molecular changes involving changes in glucose metabolism leading to generation of advanced glycation end products (AGEs), changes in *O*-glycosylation of cytosolic and nuclear proteins, activation of protein kinase C and increased expression of reactive oxygen species and TGF- β [7]. All these changes contribute to the development of diabetic nephropathy, which in turn can lead to end-stage renal disease (ESRD), where dialysis and kidney transplantation are treatment options. Kidney transplantation improves patient survival compared with long-term dialysis [8,9].

Further treatment options for patients with diabetic ESRD also include pancreatic transplantation [10], and these patients can receive simultaneous pancreas and kidney (SPK) or kidney transplants alone (KA). Several studies demonstrate improved survival and reduced cardiovascular events in SPK transplants compared with KA recipients [11–13], but long-term follow-up studies are needed to demonstrate possible differences in effects of SPK transplantation versus kidney transplantation alone [14]. Recurrence of diabetic allograft nephropathy might also be prevented (References listed in [15]). Furthermore, improved glycemic control as a result of pancreatic transplantation may lead to lowered risk of cardiovascular disease, both in patients with type 1 and type 2 diabetes [16,17].

The importance of strict blood glucose control to decrease the risk of long-term complications has been well documented in large clinical trials, both for patients with type 1

and type 2 diabetes [3,16,18]. The importance of inflammation in development of complications has been addressed [6,19] as has also the long-term effects of endothelial dysfunction [20]. Furthermore, the importance of changes in the extracellular matrix (ECM) in the kidney glomeruli, mesangial matrix and interstitium has also been studied in relation to changes in kidney function in diabetes [4].

Proteoglycans (PGs) are essential structural and functional components of the ECM. PGs are proteins substituted with long sugar chains – glycosaminoglycans (GAGs) – able to interact with a range of partner molecules, and being essential for many of the functions of the PGs. The extent and functional consequences of such interactions are affected by the structural composition and degree of post-translational modifications of the sugar entities, including sulfation. One particular ECM component, heparan sulfate (HS) PGs, has been the focus of several studies. Chondroitin sulfate (CS)/dermatan sulfate (DS) PGs however, have been studied only to a limited extent [4]. A series of studies have supported the notion that changes in the amount and structures of HSPGs may lead to lower charge density in the basement membranes of the kidneys, thereby contributing to proteinuria [21]. However, some recent studies have provided conflicting data and challenged this hypothesis. This issue has been reviewed and the complexity of the regulation of kidney filtration suggest that HSPGs may be one of several factors involved in the development of albuminuria in diabetes [7,22].

The purpose of this study was to investigate the long term effects of hyperglycemia on inflammatory parameters and on ECM and PG structures important for renal filtration. Our results suggest improved inflammatory and metabolic profiles in the normoglycemic SPK recipients. In kidneys, differences in basement membrane thickness and distinct HSPG structures were observed.

2. Methods

2.1. Ethics statement

Experiments and tests were approved by the regional ethics committee for Southern Norway, and followed the guidelines of the Helsinki declaration. All participants gave written consent for their participation.

2.2. Patients and study design

Twenty-seven patients with type 1 diabetes and former ESRD leading to kidney transplantation (Table 1) were included at Oslo University Hospital, Rikshospitalet, Department of Transplant Medicine, Section of Nephrology. This is a subgroup of a larger study, used for a targeted investigation of inflammatory and ECM parameters. Data, blood samples and kidney biopsies were collected 9 or more years after transplantation, from patients with functional grafts only.

This study is a cross-sectional single-center study comprising follow-up data on patients attending the only hospital performing transplantations in Norway. Surgical procedures were done by state-of-the art techniques in all included patients. Only patients with functional grafts were included in the study. The local nephrologists referred the patients to the two

different types of treatment based on whether the patient had a live kidney donor available or not. At the time of patient inclusion the scientific knowledge on SPK versus KA transplantation was limited. Patients with significantly elevated C-reactive protein (CRP)-levels were further investigated by clinical journal assessments. Use of antibiotics, reports on clinical suspicion of infections or elevated white blood cells counts in additions to elevated CRP at the time of blood sampling, led to exclusion from this actual part of the patient study.

17 patients received a combined pancreas and kidney allograft (SPK) and 10 patients received a kidney transplant alone (KA) from a live donor, preferentially a family related donor. All patients received a calcineurin inhibitor (CNI)-based immunosuppressive regimen with addition of 5 mg of prednisolone o.d. Most patients also received an antiproliferative agent, being either mycophenolate or azathioprine. In the KA group 6 patients used cyclosporine and 4 patients used tacrolimus. Six out of 10 patients were treated with angiotensin converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). In the SPK group 5 used cyclosporine and 12 patients used tacrolimus. Only 6 out of 17 patients received either ACE inhibitors or ARBs. Patient characteristics are further presented in Section 3 and Tables 1 and 2.

2.3. Inflammatory mediator- and ECM turnover assays

C-reactive protein (CRP) levels in patient blood were determined in the clinic by the routine laboratory.

Blood samples were collected in EDTA and heparin coated tubes. The EDTA coated tubes were kept on ice, while the heparin coated tubes were kept at room temperature, both for a maximum of 2 h. Plasma was obtained by centrifugation (13,000 rpm, 4 °C, and 12 min). 100 µl 0.1 M HEPES and 100 µl of 0.1 M CaCl₂ was added to each 1 ml of plasma from the heparinized blood, and stored at -75 °C.

The levels of matrix metalloproteinase (MMP)-9, MMP-2, tissue inhibitor of metalloproteinase (TIMP)-1, TIMP-2, intracellular cell adhesion molecule (ICAM)-1, vascular CAM (VCAM)-1, vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF/CCN2) and TGF-β (in plasma from heparinized blood) and plasminogen activator inhibitor (PAI)-1 (plasma from blood collected into EDTA coated tubes) were analyzed by enzyme-linked immunoassays (ELISAs) from R&D Systems, following the manufacturer's instructions.

Cytokines and chemokines were measured in plasma from the EDTA coated tubes by a luminex antibody kit (BioRad Laboratories) for a panel of 8 cytokines and chemokines: tumor necrosis factor (TNF) α, interleukin (IL)-1β, IL-6, CXCL8, IL-10, CCL2, CCL3 and CCL4.

2.4. Lipid measurements

Triglycerides, total cholesterol, HDL and LDL were measured by standard clinical assays. To prevent oxidation, argon gas was added to plasma containing EDTA and samples were thereafter immediately frozen at -75 °C. The amount of free fatty acids (FFA)/non-esterified

fatty acids (NEFA) in serum from all patients included were quantified on the MaxMatPL system using the NEFA kit from Dialab, Wiener Neudorf, Austria.

2.5. Mesangial matrix and basement membrane

Kidney graft biopsies were fixed in 2% (w/v) glutaraldehyde (Agar Scientific, Stansted, Essex, UK) in 0.1 M PBS, pH 7.4. The samples were dehydrated in graded alcohol during progressive lowering of the temperature and embedded in a methacrylate resin [23]. Semithin sections were cut from coded blocks and stained with toluidine blue for orientation. Areas containing at least two glomeruli were subjected to ultrathin sectioning followed by uranyl acetate/lead citrate contrasting. Low power electron micrographs were used for calculation of volume density of the mesangium in 7 hyperglycemic patients with HbA1c > 6.5% (48 mmol/mol) and 8 normoglycemic patients with HbA1c < 6.5% (48 mmol/mol). The morphometry was performed by point-counting using semiautomatic interactive image analysis (AnalySIS, Soft Imaging System, Münster, Germany). At medium power magnification the thickness of basement membranes was measured from the five thinnest areas of five chosen thin capillary loops using the same software in samples from three normoglycemic and five hyperglycemic patients.

2.6. Glycosaminoglycan (GAG) extraction from human kidney biopsies

Glomeruli-rich kidney graft biopsies from the same patients used for measurements of basement membrane thickness was kept in PBS at 4 °C for 1 h before air-dried and stored at -80 °C until analysis. After lyophilization, samples of dry weight less than 1 mg were suspended in 1 ml protein digestion buffer (1 mM CaCl₂, 1% Triton X-100, 50 mM Tris/HCl, pH 8.0) containing 0.5 mU protease from *Streptomyces griseus* (Sigma) and incubated overnight at 55 °C with end-over-end mixing. DNA was degraded using benzonase (Sigma) digestion at 37 °C for 3 h after heat inactivation of protease. GAGs were then released from core proteins by β -elimination reaction (0.5 M NaOH, end-over-end mixing overnight at 4 °C). The sample solution was neutralized to pH 6.0 with formic acid and diluted with 2–3 ml distilled water. The diluted sample solution was applied to a DEAE (Sigma)-Sephacrose column (1.4 ml), and GAGs were eluted with 3 volumes of 1 M NaCl, 20 mM NaOAc, pH 6.0. Finally, the purified GAGs were desalted using PD-10 columns (GE Health Life Science). The eluates were divided into seven equal aliquots and dried. Three aliquots were used for heparin lyase digestion and three for chondroitinase digestion.

2.7. Disaccharide analysis

Both HS and CS disaccharides were prepared from the purified GAG samples. For HS disaccharides, 30 μ l HS digestion buffer (5 mM Ca(OAc)₂, 20 mM Tris/HCl pH7.4) containing 100 mU heparin lyase I (New England Biolabs), 10 mU heparin lyase II, and 10 mU heparin lyase III (Both a generous gift from Jian Liu, University of North Carolina Eshelman School of Pharmacy, Chapel Hill, NC, USA) was added to dried purified GAG samples. The solution was incubated at 37 °C for 5 h and a second aliquot of 100 mU heparin lyase I, 10 mU heparin lyase II, and 10 mU heparin lyase III was added for overnight incubation at 37 °C. For CS disaccharides, 30 μ l CS digestion buffer (5 mM

NH₄OAc, 25 mM Tris/HCl pH8.0) containing 20 mU chondroitinase ABC (Sigma) was added to dried GAG samples. The sample solution was incubated overnight at 37 °C.

HS and CS derived disaccharides were measured by size exclusion chromatography–mass spectrometry (SEC–MS), as previously described [24].

2.8. Statistical analyses

Data were analyzed using GraphPad Prism 5.03. Results are presented as mean ± standard error of the mean (SEM). Differences between the groups were assessed using the non-parametrical two-tailed Mann Whitney *U*-test. *P*-values less than 0.05 were considered to indicate significance.

3. Results

Patients participating in this study had similar duration of type 1 diabetes at transplantation and were also comparable in age at time of transplantation. The majority of both SPK (15 out of 17) and KA (7 out of 10) were men. There was no major difference in systolic or diastolic blood pressure, while BMI was significantly higher in the KA group ($p = 0.02$, Table 1). No differences in kidney markers were observed. Notably, the KA group, still in need of insulin injections, had significantly higher HbA1c levels than the SPK recipients ($8.6 \pm 0.6\%$ (70 ± 7 mmol/mol) vs $5.8 \pm 0.1\%$ (40 ± 1 mmol/mol), $p < 0.0001$). These patients also had significantly higher CRP levels (3.7 ± 1.6 vs 1.3 ± 0.4 , $p = 0.023$), reflecting a higher degree of inflammation.

To address the implications of hyperglycemia on inflammatory mediators in these patients, plasma from both groups were analyzed for both pro- and anti-inflammatory cytokines and chemokines. From Table 2 it is evident that there were large individual variations and no major differences between the two groups with regard to the classic pro-inflammatory mediators TNF α or IL-6. Chemokine levels were also similar, and the pro-inflammatory IL- β and anti-inflammatory IL-10 were below detection limits. The only exception was CCL4 which was significantly higher in the SPK group ($p = 0.018$).

Persons with type 1 diabetes have increased risk of cardiovascular disease, and plasma lipid parameters are important indicators used to evaluate this risk. In this study there was no difference in the concentrations of total cholesterol, LDL, HDL and free fatty acids. However, the level of triglycerides was significantly lower in the SPK than in the KA group ($p = 0.01$, Table 2).

Development of fibrosis in diabetic nephropathy has been linked to elevated levels of the pro-fibrotic growth factor TGF- β and CTGF/CCN2 [6]. The average concentrations of both these factors were not different between the two groups (Table 3). However, the levels of growth factor VEGF, linked to endothelial dysfunction and renal filtration, were significantly higher in the KA group ($p = 0.007$). This was also the case for PAI-1 ($p = 0.023$). PAI-1 may be linked both to changes in ECM turnover and inflammation in diabetes as well as renal fibrosis [25].

The link between inflammation and changes in ECM turnover can also be addressed by measuring levels of MMPs and the TIMPs, their corresponding inhibitors. MMP-9 in particular, is involved in inflammatory and atherosclerotic processes, and is expressed by important immune cells like granulocytes and monocytes [26]. Whether there are differences in the MMP-9 levels between the KA and SPK group remains inconclusive. There was no difference in the concentrations of MMP-2 and its corresponding inhibitor TIMP-2. Cell adhesion molecules and cell surface HSPGs are important players in the recruitment of leucocytes in inflammatory and atherosclerotic processes. In both groups the levels of ICAM and VCAM were approximately the same. However, the concentration of the shedded cell surface HSPG syndecan-1 was 42% higher in the KA group compared to the SPK group, although this change did not reach statistical significance ($p = 0.087$, Table 2).

Increased glomerular basement membrane thickness and mesangial matrix volume is related to diabetic nephropathy [27]. When comparing our diabetic (HG) to non-diabetic (NG) participants, as judged by HbA1c, we found that the average basement membrane thickness was significantly increased in the group with diabetes ($p = 0.05$, Table 3). However, no difference in mesangial matrix volumes was observed (Table 3).

Changes in ECM structure and composition are major features of diabetic nephropathy. PGs are important parts of glomerular basement membranes, and structural alterations of both HS and CS GAG chains covalently attached to different PGs could be related to filtration changes of diabetic nephropathy. To investigate further the implications of possible structural changes of the renal PG species in relation to hyperglycemia, HS and CS/DS GAGs were extracted from kidney biopsies from the same individuals analyzed for glomerular basement thickness and mesangial matrix volume. Results presented in Table 3 show the disaccharide composition of both the HS and CS/DS in material from both groups, determined using size exclusion chromatography–mass spectrometry (SEC–MS). Only minor amounts of DS could be detected based on chondroitinase digestions. The majority of the CS disaccharides was of the mono-sulfated species and did not differ between the groups. For the HS-chains however, differences in composition between the two groups were observed. The fraction of the *N*-acetylated, singly sulfated D0A6/D2A0 disaccharides were significantly higher in HG material ($p = 0.05$), while the *N*-sulfated D0S0 was correspondingly, but not significantly, decreased. In addition, a significant decrease in the 6-*O*-sulfated D2S6 species was observed in the HG patients ($p = 0.05$).

4. Discussion

The SPK group was normoglycemic, whereas the KA group was hyperglycemic, with an average HbA1c of 5.8% (40 mmol/mol) and 8.6% (70 mmol/mol) respectively. The risk of long-term complications in patients with diabetes is related to glycemic control [3,16,18,28]. Thus, these groups offer a unique opportunity to study the impact of hyperglycemia on inflammatory and metabolic parameters, as well as ECM kidney parameters.

The SPK group had significantly lower levels of CRP, PAI-1 and VEGF, parameters related to inflammation, ECM turnover and endothelial dysfunction, when compared with KA patients. CRP levels, normally reflecting acute phase responses, have been shown to be

elevated in patients suffering from long-term type 1 diabetes, compared to patients with newly onset diabetes and healthy controls [29]. This may be a sign of ongoing low-grade inflammatory processes, but the functional consequences of elevated CRP levels have not been documented. While CRP is an established clinical marker of infection and inflammation, PAI-1 is associated with diabetic nephropathy and has pro-fibrotic properties due to its ability to prevent plasmin and MMP activation and activate TGF- β [25]. PAI-1 is involved in regulation of coagulation and considered a risk factor for cardiovascular disease and associated also with metabolic syndrome [30]. VEGF is considered an important contributor to diabetic complications [6]. Experimental studies suggest that use of antibodies against VEGF can be renoprotective [31], but interfering with VEGF activities may also affect renal structures [32]. However, pro-VEGF treatment has been shown to reduce the risk of macrovascular complications in diabetes [33], highlighting the fact that a balanced level of inflammatory mediators is a key to maintain homeostasis. VEGF may be a potential future target for intervention in order to reduce diabetic complications. CCL4, increased in the SPK-group, is regarded as a classic pro-inflammatory mediator [34]. However, this chemokine is involved in suppression of inflammatory response in pancreas of nonobese diabetic mice after IL-4 treatment [35], suggesting importance also in anti-inflammatory reactions. Our further data generated on other chemokines and cytokines did not allow for conclusions due to individual variations.

The SPK group showed a more favorable metabolic profile, judged from the parameters HbA1c, BMI, PAI-1 and triglycerides. These findings could reflect the positive effects of the pancreatic transplantation with functioning insulin secretion and the down-stream insulin effects on blood glucose regulation and metabolism in general. This is supported by the recent study from a larger cohort in this patient group, demonstrating improved survival in the SPK group [11]. However, the improved inflammatory profile demonstrated can also contribute to the positive effects of pancreas transplantation. Low grade inflammation and endothelial dysfunction are both factors that may contribute to long-term complications in both type 1 and type 2 diabetes [6]. Hence, an improved profile in the SPK patients for factors involved in these processes are further positive contributors to the improved survival [11].

Microalbuminuria is an early clinical marker of kidney affection in diabetes [36,37], and changes in kidney ECM contribute to altered kidney filtration [4]. Kidney basement membranes are important for filtration [38] and increase in glomerular basement membrane thickness is related to impaired renal filtration [4]. In line with this, we found that the glomerular basement membranes were thicker in the KA group. PGs are important components of the basement membranes [39], but the roles of PGs in renal filtration are debated. Altered HS expression and sulfation is shown in both animal models and human material [40–43], but this is challenged by others [44,45]. In this study we demonstrate alterations in distinct HSPG structures in hyperglycemia. The increase in non-*N*-sulfated disaccharides (D0A6 and D2A0) and the corresponding decrease in *N*-sulfation (D0S0) suggest an effect on *N*-sulfation. Further, we demonstrate a significant decrease in the 6-*O*-sulfated D2S6 disaccharide. HS fine structure is fundamental for interactions with partner molecules including inflammatory cytokines and growth factors. 6-*O*-sulfation has proved to

be of particular importance for several such interactions, also in renal fibrosis [46], therefore this distinct change in HS structure in the kidney after prolonged hyperglycemia is of considerable interest. In addition to regulation at the biosynthetic level, 6-*O*-sulfation is also regulated at the post-transcriptional level by the action of Sulf. In line with our observations, Sulf expression is increased in diabetes [27,47]. HSPGs are shedded from the cell surface by MMPs, and shedding of HSPG syndecan-1 is increased with microalbuminuria in type-1 diabetes [48]. In this material syndecan-1 shedding tended to be increased in the KA patients, further suggesting syndecan-1 as a marker for early kidney changes in diabetes. While HSPGs are the dominating species in kidney, CSPGs are expressed to a smaller degree, and changes in these structures might not be obvious until more overt changes in kidney functions take place. Notably, in diabetic mouse kidney we have observed a reduced sulfation of CS but not HS [49].

In this study we present data on patients with diabetes and with KA or SPK transplants, and verify the better glycemic control of the latter group. Our results supports the long term effects of hyperglycemia by presenting reduced inflammatory activities and healthier metabolic profiles 9–12 years after SPK transplantations, as well as effects on basement membrane thickness and heparan sulfate structure in the kidney. How to improve these parameters in patients only receiving KA transplants is a challenge of further research.

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REFERENCES

1. van Hoven KH, Factor SM. Diabetic heart disease: the clinical and pathological spectrum—Part I. *Clin Cardiol.* 1989; 12:600–604. [PubMed: 2680199]
2. Astorri E, Fiorina P, Gavaruzzi G, Astorri A, Magnati G. Left ventricular function in insulin-dependent and in non-insulin-dependent diabetic patients: radionuclide assessment. *Cardiology.* 1997; 88:152–155. [PubMed: 9096915]
3. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med.* 1993; 329:977–986. [PubMed: 8366922]
4. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. *J Histochem Cytochem.* 2012; 60:976–986. (Official Journal of the Histochemistry Society). [PubMed: 23103723]
5. Brownlee M. The pathobiology of diabetic complications. *Diabetes.* 2005; 54:1615–1625. [PubMed: 15919781]
6. Forbes JM, Cooper ME. Mechanisms of diabetic complications. *Physiol Rev.* 2013; 93:137–188. [PubMed: 23303908]
7. Kanwar YS, Sun L, Xie P, Liu F-y, Chen S. A glimpse of various pathogenetic mechanisms of diabetic nephropathy. *Annu Rev Pathol: Mech Dis.* 2011; 6:395–423.
8. Schnuelle P, Lorenz D, Trede M, Van Der Woude FJ. Impact of renal cadaveric transplantation on survival in end-stage renal failure: evidence for reduced mortality risk compared with hemodialysis during long-term follow-up. *J Am Soc Nephrol.* 1998; 9:2135–2141. [PubMed: 9808102]

9. Wolfe RA, Ashby VB, Milford EL, Ojo AO, Ettenger RE, Agodoa LY, et al. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. *N Engl J Med*. 1999; 341:1725–1730. [PubMed: 10580071]
10. Gruessner AC. 2011 Update on pancreas transplantation: comprehensive trend analysis of 25,000 cases followed up over the course of twenty-four years at the International Pancreas Transplant Registry (IPTR). *Rev Diabetic Stud: RDS*. 2011; 8:6–16.
11. Lindahl JP, Hartmann A, Horneland R, Holdaas H, Reisaeter AV, Midtvedt K, et al. Improved patient survival with simultaneous pancreas and kidney transplantation in recipients with diabetic end-stage renal disease. *Diabetologia*. 2013; 56:1364–1371. [PubMed: 23549518]
12. Perseghin G, Fiorina P, De Cobelli F, Scifo P, Esposito A, Canu T, et al. Cross-sectional assessment of the effect of kidney and kidney-pancreas transplantation on resting left ventricular energy metabolism in type 1 diabetic-uremic patients: a phosphorous-31 magnetic resonance spectroscopy study. *J Am Coll Cardiol*. 2005; 46:1085–1092. [PubMed: 16168295]
13. La Rocca E, Fiorina P, Astorri E, Rossetti C, Lucignani G, Fazio F, et al. Patient survival and cardiovascular events after kidney-pancreas transplantation: comparison with kidney transplantation alone in uremic IDDM patients. *Cell Transplant*. 2000; 9:929–932. [PubMed: 11202580]
14. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M. Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med*. 1998; 339:69–75. [PubMed: 9654536]
15. Lindahl JP, Jenssen T, Hartmann A. Long-term outcomes after organ transplantation in diabetic end-stage renal disease. *Diabetes Res Clin Pract*. 2014; 105:14–21. [PubMed: 24698407]
16. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med*. 2005; 353:2643–2653. [PubMed: 16371630]
17. Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*. 2008; 359:1577–1589. [PubMed: 18784090]
18. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). *Lancet*. 1998; 352:854–865. [PubMed: 9742977]
19. Navarro JF, Mora C. Role of inflammation in diabetic complications. *Nephrol Dial Transplant*. 2005; 20:2601–2604. (Official Publication of the European Dialysis and Transplant Association—European Renal Association). [PubMed: 16188894]
20. Salmon AHJ, Satchell SC. Endothelial glycocalyx dysfunction in disease: albuminuria and increased microvascular permeability. *J Pathol*. 2012; 226:562–574. [PubMed: 22102407]
21. Kanwar YS, Danesh FR, Chugh SS. Contribution of proteoglycans towards the integrated functions of renal glomerular capillaries: a historical perspective. *Am J Pathol*. 2007; 171:9–13. [PubMed: 17591948]
22. Harvey SJ, Miner JH. Revisiting the glomerular charge barrier in the molecular era. *Curr Opin Nephrol Hypertens*. 2008; 17:393–398. [PubMed: 18660676]
23. Hultenby K, Reinholt FP, Oldberg A, Heinegård D. Ultrastructural immunolocalization of osteopontin in metaphyseal and cortical bone. *Matrix*. 1991; 11:206–213. [PubMed: 1870452]
24. Shi X, Zaia J. Organ-specific heparan sulfate structural phenotypes. *J Biol Chem*. 2009; 284:11806–11814. [PubMed: 19244235]
25. Lee HB, Ha H. Plasminogen activator inhibitor-1 and diabetic nephropathy. *Nephrology*. 2005; 10:S3–S11.
26. Opdenakker G, Van den Steen PE, Dubois B, Nelissen I, Van Coillie E, Masure S, et al. Gelatinase B functions as regulator and effector in leukocyte biology. *J Leukocyte Biol*. 2001; 69:851–859. [PubMed: 11404367]
27. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. *J Histochem Cytochem*. 2012; 60:976–986. [PubMed: 23103723]
28. Daneman D. Type 1 diabetes. *Lancet*. 2006; 367:847–858. [PubMed: 16530579]
29. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N Engl J Med*. 2005; 353:2643–2653. [PubMed: 16371630]

30. Alessi MC, Juhan-Vague I. Metabolic syndrome, haemostasis and thrombosis. *Thromb Haemost.* 2008; 99:995–1000. [PubMed: 18521499]
31. de Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol.* 2001; 12:993–1000. [PubMed: 11316858]
32. Advani A, Kelly DJ, Advani SL, Cox AJ, Thai K, Zhang Y, et al. Role of VEGF in maintaining renal structure and function under normotensive and hypertensive conditions. *Proc Nat Acad Sci USA.* 2007; 104:14448–14453. [PubMed: 17726104]
33. Duh E, Aiello LP. Vascular endothelial growth factor and diabetes: the agonist versus antagonist paradox. *Diabetes.* 1999; 48:1899–1906. [PubMed: 10512352]
34. Maurer M, von Stebut E. Macrophage inflammatory protein-1. *Int J Biochem Cell Biol.* 2004; 36:1882–1886. [PubMed: 15203102]
35. Meagher C, Arreaza G, Peters A, Strathdee CA, Gilbert PA, Mi QS, et al. CCL4 protects from type 1 diabetes by altering islet beta-cell-targeted inflammatory responses. *Diabetes.* 2007; 56:809–817. [PubMed: 17327452]
36. Parving HH, Oxenboll B, Svendsen PA, Christiansen JS, Andersen AR. Early detection of patients at risk of developing diabetic nephropathy. A longitudinal study of urinary albumin excretion. *Acta Endocrinol.* 1982; 100:550–555. [PubMed: 6812342]
37. Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet.* 1982; 1:1430–1432. [PubMed: 6123720]
38. Farquhar MG, Wissig SL, Palade GE. Glomerular permeability. I. Ferritin transfer across the normal glomerular capillary wall. *J Exp Med.* 1961; 113:47–66. [PubMed: 13698249]
39. Kanwar YS, Farquhar MG. Presence of heparan sulfate in the glomerular basement membrane. *Proc Nat Acad Sci USA.* 1979; 76:1303–1307. [PubMed: 155819]
40. Raats CJ, Van Den Born J, Berden JH. Glomerular heparan sulfate alterations: mechanisms and relevance for proteinuria. *Kidney Int.* 2000; 57:385–400. [PubMed: 10652015]
41. van den Born J, van den Heuvel LP, Bakker MA, Veerkamp JH, Assmann KJ, Berden JH. A monoclonal antibody against GBM heparan sulfate induces an acute selective proteinuria in rats. *Kidney Int.* 1992; 41:115–123. [PubMed: 1593846]
42. Wijnhoven TJ, Lensen JF, Rops AL, van der Vlag J, Kolset SO, Bangstad HJ, et al. Aberrant heparan sulfate profile in the human diabetic kidney offers new clues for therapeutic glycomimetics. *Am J Kidney Dis.* 2006; 48:250–261. [PubMed: 16860191]
43. Yard BA, Kahlert S, Engelleiter R, Resch S, Waldherr R, Groffen AJ, et al. Decreased glomerular expression of agrin in diabetic nephropathy and podocytes, cultured in high glucose medium. *Exp Nephrol.* 2001; 9:214–222. [PubMed: 11340306]
44. Harvey SJ, Jarad G, Cunningham J, Rops AL, van der Vlag J, Berden JH, et al. Disruption of glomerular basement membrane charge through podocyte-specific mutation of agrin does not alter glomerular permselectivity. *Am J Pathol.* 2007; 171:139–152. [PubMed: 17591961]
45. van den Born J, Pisa B, Bakker MA, Celie JW, Straatman C, Thomas S, et al. No change in glomerular heparan sulfate structure in early human and experimental diabetic nephropathy. *J Biol Chem.* 2006; 281:29606–29613. [PubMed: 16885165]
46. Alhasan AA, Spielhofer J, Kusche-Gullberg M, Kirby JA, Ali S. Role of 6-O-sulphated heparan sulphate in chronic renal fibrosis. *J Biol Chem.* 2014; 289:20295–20306. [PubMed: 24878958]
47. Schumacher VA, Schlötzer-Schrehardt U, Karumanchi SA, Shi X, Zaia J, Jeruschke S, et al. WT1-dependent sulfatase expression maintains the normal glomerular filtration barrier. *J Am Soc Nephrol.* 2011; 22:1286–1296. [PubMed: 21719793]
48. Svennevig K, Kolset SO, Bangstad HJ. Increased syndecan-1 in serum is related to early nephropathy in type 1 diabetes mellitus patients. *Diabetologia.* 2006; 49:2214–2216. [PubMed: 16832664]
49. Reine TM, Grondahl F, Jenssen TG, Hadler-Olsen E, Prydz K, Kolset SO. Reduced sulfation of chondroitin sulfate but not heparan sulfate in kidneys of diabetic db/db mice. *J Histochem Cytochem.* 2013; 61:606–616. (Official Journal of the Histochemistry Society). [PubMed: 23757342]

Table 1

Patient characteristics and clinical markers.

Characteristics	KA (<i>n</i> = 10)	SPK (<i>n</i> = 17)	<i>P</i> value
Age at tx (yrs)	43.3 ± 3.7	42.2 ± 1.4	0.782
Age at follow-up (yrs)	52.23 ± 3.6	53.8 ± 1.5	0.670
Follow-up time (yrs)	8.9 ± 0.4	11.6 ± 1.1	0.056
Female/male	3/7	2/15	–
Yrs w/diabetes at tx	31.0 ± 3.8	30.9 ± 1.8	0.688
BMI (kg/(m ²))	27.9 ± 1.6	23.4 ± 0.7*	0.020
SBP (mmHg)	136.0 ± 4.8	143.6 ± 2.2	0.227
DBP (mmHg)	78.9 ± 3.9	74.1 ± 2.0	0.326
HbA1c (% (mmol/mol))	8.6 ± 0.6 (70 ± 7)	5.8 ± 0.1 (40 ± 1) ***	<0.0001
Hb (g/dl)	12.9 ± 0.4	13.8 ± 0.4	0.092
CRP (mg/l)	3.7 ± 1.6	1.3 ± 0.4 *	0.023
eGFR baseline (MDRD)	66.30 ± 3.754	69.00 ± 2.442	0.580
GFR (isotope) at follow-up	52.6 ± 2.4	59.0 ± 4.4	0.507
Creatinine (μmol/l)	121.4 ± 9.1	109.6 ± 7.7	0.422
Urea (mmol/l)	11.3 ± 1.0	8.9 ± 0.7	0.063
Fasting C-peptide (pmol/l)	0.0 ± 0.0	0.8 ± 0.1 ***	<0.0001
ALAT (U/l)	25.4 ± 4.6	21.8 ± 4.6	0.247

Data are presented as mean ± SEM.

* *p*-Value 0.05,*** *p*-Value 0.001.

Tx, transplantation; yrs, years; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin A1c, is presented as NGSP units (%) and with IFCC units (mmol/mol) in parentheses. ALAT, alanine transaminase. All values but eGFR baseline (3 months after tx) are from the follow-up.

Table 2

Lipid parameters and mediators in inflammation and extracellular matrix turnover.

	KA	SPK	<i>p</i> Value
TNF α (pg/ml)	18.63 $\pm$ 11.64	36.41 $\pm$ 17.61	0.281
IL-6 (pg/ml)	7.415 $\pm$ 1.762	8.038 $\pm$ 2.918	0.365
CXCL8 (pg/ml)	7.289 $\pm$ 2.372	5.084 $\pm$ 0.398	1.000
CCL2 (pg/ml)	22.22 $\pm$ 3.889	23.27 $\pm$ 2.111	0.510
CCL4 (pg/ml)	27.38 $\pm$ 3.523	39.22 $\pm$ 2.842*	0.018
TGF- β (pg/ml)	8248 $\pm$ 1764	6556 $\pm$ 1426	0.236
CTGF/CCN2 (pg/ml)	484.1 $\pm$ 47.29	425.3 $\pm$ 52.12	0.236
VEGF (pg/ml)	49.75 $\pm$ 7.245	25.02 $\pm$ 3.439**	0.007
MMP-9 (ng/ml)	47.05 $\pm$ 7.828	38.42 $\pm$ 3.052	0.598
TIMP-1 (ng/ml)	141.0 $\pm$ 9.992	129.0 $\pm$ 5.762	0.360
PAI-1 (ng/ml)	5.002 $\pm$ 1.230	1.877 $\pm$ 0.418*	0.023
Syndecan-1 (ng/ml)	60.20 $\pm$ 8.926	42.32 $\pm$ 5.612	0.087
ICAM-1 [#] (ng/ml)	214.1 $\pm$ 26.18	184.8 $\pm$ 15.60	0.815
VCAM-1 [#] (pg/ml)	219.1 $\pm$ 15.27	228.3 $\pm$ 26.04	0.815
MMP-2 ^{##} (ng/ml)	204.1 $\pm$ 13.59	209.6 $\pm$ 14.73	0.798
TIMP-2 ^{##} (ng/ml)	90.69 $\pm$ 5.733	93.87 $\pm$ 5.294	0.798
Cholesterol (mmol/l)	5.060 $\pm$ 0.292	4.388 $\pm$ 0.230	0.064
LDL cholesterol (mmol/l)	2.730 $\pm$ 0.177	2.400 $\pm$ 0.176	0.256
HDL cholesterol (mmol/l)	1.570 $\pm$ 0.167	1.600 $\pm$ 0.100	0.560
Triglycerides (mmol/l)	1.680 $\pm$ 0.203	0.900 $\pm$ 0.094**	0.002
FFA (mmol/l)	0.582 $\pm$ 0.085	0.525 $\pm$ 0.074	0.412

Data are presented as mean $\pm$ SEM.* *p*-Value 0.05.** *p*-Value 0.01. *n* = 10 (KA), 17 (SPK).# *n* = 8 (KA), 9 (SPK).## *n* = 8 (KA), 8 (SPK).

Table 3

Basement membrane thickness, mesangial matrix volume and disaccharide structures in kidney biopsies.

	Hyperglycemic	Normoglycemic	P value
GMB thickness (nm)	424.6 ± 43.64	234.3 ± 10.59*	0.0357
Mesangial matrix volume (RU) [#]	0.181 ± 0.027	0.179 ± 0.023	0.9551
<i>HS disaccharides (%)</i>			
D2S6	2.020 ± 0.088	3.216 ± 0.232*	0.0357
D2A6	0.078 ± 0.008	0.130 ± 0.036	0.1529
D2S0/D0S6	18.89 ± 0.361	19.43 ± 1.712	1.0000
D0A6/D2A0	23.87 ± 0.386	20.60 ± 1.576*	0.0357
D0S0	17.19 ± 0.277	20.78 ± 3.610	0.7857
D0A0	37.95 ± 0.612	35.90 ± 0.834	0.2500
<i>CS disaccharides (%)</i>			
D2a6/D2a4/D0a10	9.248 ± 0.335	7.225 ± 1.385	0.1905
D0a4/D0a6/D2a0	89.33 ± 0.381	91.88 ± 1.180	0.0952
D0a0	1.428 ± 0.123	0.895 ± 0.205	0.1143

Data are presented as mean ± SEM.

* *p*-Value 0.05, *n* = 3 (HG), 5 (NG).

[#] *n* = 7 (HG), 8 (NG).

Disaccharide notations used for HS: D0A0 = UA-GlcNAc, D0S0 = UA-GlcNS, D0A6 = UA-GlcNAc6S, D2A0 = UA2S-GlcNAc, D0S6 = UA-GlcNS6S, D2S0 = UA2S-GlcNS, D2A6 = UA2S-GlcNAc6S and D2S6 = UA2S-GlcNS6S, and disaccharide notations used for CS: D0a0 = UA-GalNAc, D0a4 = UA-GalNAc4S, D0a6 = UA-GalNAc6S, D0a10 = UA-GalNAc4S6S, D2a0 = UA2S-GalNAc, D2a4 = UA2S-GalNAc4S, D2a6 = UA2S-GalNAc6S. UA is the 4,5-unsaturated uronic acid generated during the enzymatic degradation.