PAGE 1: TITLE PAGE

Visceral fat is strongly associated with post-transplant diabetes mellitus and glucose metabolism one year after kidney transplantation

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DISCLOSURES

The authors of this manuscript have no conflicts of interest to disclose.

PAGE 2: AUTHORSHIP PAGE

AUTHOR CONTRIBUTIONS

MEVD participated in planning of the study and study design, acquisition of data, analyzed and interpreted data and writing the manuscript.

AH, TJ, JB, AÅ contributed to the study design, contributed to the acquisition of data, and editing of the manuscript.

KG contributed to the acquisition of data.

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PAGE 3: ABBREVIATIONS PAGE

DXA: Dual-energy x-ray absorptiometry

fPG: Fasting plasma glucose

HOMA-IR index: Homeostatic Model Assessment-insulin resistance index

HOMA-B%: Homeostatic Model Assessment-beta cell function

IFG: Impaired fasting glucose

IGT: Impaired glucose tolerance

IR: Insulin Resistance

NGT: Normal glucose tolerance

OGTT: Oral glucose tolerance test

PTDM: Post transplant diabetes mellitus

VAT: Visceral adipose tissue

$VAT_{\%totBFM:}$ Percentage visceral adipose tissue of total body fat mass

2hPG: 2-hour plasma glucose

PAGE 4: CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been previously published in whole or part. Some of the data have been published in abstract format presented in june 2016 during the American Transplant Congress in Boston, USA.

None of the authors have any conflicts to declare.

Body composition after kidney transplantation is linked to glucose metabolism, and impaired glucose metabolism is associated with increased risk of cardiovascular events and death. One year after transplantation we examined 150 patients for new-onset diabetes performing OGTTs and body composition measurements including visceral adipose tissue (VAT) content from dual-energy X-ray absorptiometry scans. We found that glucose metabolism was generally improved and that the levels of VAT and percentage VAT of total body fat mass ($VAT_{\%totBFM}$) were lowest in those with normal glucose tolerance and highest in those with post transplant diabetes mellitus. In a multivariable linear regression analysis 87.4% of the variability in fasting glucose concentration was explained by insulin resistance ($p<0.001$,HOMA-IR index), beta cell function ($p<0.001$,HOMA-beta), $VAT_{\%totBFM}$ ($p=0.007$) and BMI ($p=0.015$) (total model $p<0.001$), while insulin resistance ($p<0.001$) and beta cell function ($p<0.001$) explained 31.9% of the variability in 2-hour glucose concentration in a multivariable model (total model $p<0.001$). VAT was associated with glucose metabolism to a larger degree than BMI. In conclusion, VAT is associated with hyperglycemia one year after kidney transplantation, and insulin resistance and beta cell function estimates are the most robust markers of glucose metabolism.

MAIN BODY

INTRODUCTION

Post transplant diabetes mellitus (PTDM) resembles type 2 diabetes, but may also be considered a separate entity with noninfectious inflammation and use of diabetogenic immunosuppressive drugs as additional provoking factors. After transplantation some degree of inflammation is unavoidable and use of immunosuppressive agents with negative impact on glucose metabolism is essential to preserve graft function and avoid rejection. Actually, choice of immunosuppressive therapy has been reported to explain up to 74% of the incidence of PTDM[1], urging the importance of close monitoring and individualization of medication. However, this does not invalidate the importance of modifiable risk factors such as energy intake exceeding energy expenditure and obesity.

Body composition and in particular abdominal obesity is linked to the development of type 2 diabetes[2-6] and also abnormal glucose metabolism after kidney transplantation[7]. Post transplant weight gain is associated with reduced graft function and graft survival[8,9] and decreased long-term survival[10]. Both impaired glucose tolerance (IGT) and PTDM are associated with adverse long-term outcomes including increased risk of cardiovascular events and death in kidney transplant recipients[11-14].

Visceral fat (VAT) is considered a marker of whole-body dysfunctional adipose tissue,[15] and is associated with insulin resistance independently of BMI[16,17]. VAT may be an interesting link between metabolic and cardiovascular disease. Until recently, VAT has been examined in patients by computed tomography (CT) scans, but with high radiation, low availability and significant costs, CT is obviously not suitable for screening of patients in a clinical setting. However, assessment of VAT has lately become feasible using novel validated software applied on standard dual-energy X-ray absorptiometry (DXA) scans[18].

We have previously presented a cross-sectional study on patients in a stable phase 8-10 weeks after kidney transplantation, showing a close association between visceral fat and glucose metabolism. Abdominal obesity and VAT were more strongly associated with

impaired fasting glucose (IFG), IGT and PTDM than body mass index (BMI) and total body fat[7]. The aim of the present paper was to assess the evolution of PTDM during the first year after kidney transplantation, and we also hypothesized that there would still be a close association between abnormalities in glucose metabolism and VAT measurements, as we found at 8-10 weeks after transplantation. The evolution in body composition and VAT during the first year post-transplant is not a a topic in the present study. In addition we wanted to assess the associations between glucose metabolism and estimates of insulin resistance and beta cell function.

PATIENTS AND METHODS

Study design and population

The study was a one year follow-up study of patients who received a kidney allograft at our transplant center from mid November 2012 to the end of 2013. All patients without known diabetes were screened by an oral glucose tolerance test (OGTT) before entering the waiting list for transplantation. Figure 1 shows the patient disposition; out of 306 transplanted adult patients in the study period, 150 non-diabetic patients had a comprehensive investigation performed both 8 (to 10) weeks that was repeated one year after transplantation. This investigation included OGTT, DXA scans and measurement of fasting plasma insulin. The study was approved by the Regional Ethics Committee for South East Norway and the study was performed according to the Helsinki declaration[19] and clinical and research activities are consistent with the Principles of the Declaration on Istanbul[20]. All patients signed a written informed consent. Demographics and transplant related data for the patient cohort is shown in Table 1. This paper presents cross-sectional data for the cohort one year post-transplant.

Glucose tolerance tests and HOMA-indices

All the examinations were performed in the morning after an overnight fast. A standard OGTT was performed with blood sampling before and 2 hours after oral ingestion of 75 g glucose. Glucose metabolism classification was according to ADA criteria for plasma glucose[21]. Normal glucose tolerance (NGT); fasting plasma glucose (fPG)<5.6 mmol/L and 2-hour plasma glucose (2hPG)<7.8 mmol/L, impaired fasting glucose (IFG); fPG 5.6-5.9 mmol/L and 2hPG<7.8 mmol/L, impaired glucose tolerance (IGT); fPG<7.0 mmol/L and 2hPG 7.8-11.0 mmol/L and post transplant diabetes mellitus (PTDM); fPG≥7.0 mmol/L or 2hPG≥11.1 mmol/L.

Plasma glucose was measured in fresh venous whole blood using a plasma calibrated glucose analyzer (Hemocue® Glucose 201, Ängelholm, Sweden, which fulfills IVD Medical Device Directive 98/79/EC)[22]. Insulin was analyzed with an enzyme-linked immunosorbent

assay kit (EIA-2935 from DRG® International, Inc., Springfield, New Jersey, USA). The homeostatic model assessment-insulin resistance (HOMA-IR) index was used as a measure of insulin resistance and calculated from fasting glucose and insulin; HOMA-IR index = (fPG (mmol/l) x fInsulin (mU/ml)) / 22.5, and the homeostatic model assessment-beta cell function (HOMA-beta) was used as a measure of beta cell function and also calculated from fasting glucose and insulin; HOMA-beta = (20 x fInsulin (mU/l)) / (fPG (mmol/l) – 3.5)[23,24]. Creatinine was analyzed with an enzymatic method on Modular P (Roche Diagnostics, Pleasanton, CA, USA). All patients were routinely recommended started or continued on statin treatment after 3 months post transplant.

Body Composition

Total body composition was determined by DXA using a narrow fan-beam Lunar Prodigy Densitometer (GE Healthcare) and all the scans were analyzed using enCORE^{R} software version 14.10 (GE Medical Systems, Lunar Corp., Madison, WI)[3]. Two certified densitometry technologists performed all scans. The VAT quantification was manually performed by one operator, using the enCORE® software. This operator showed an intra individual coefficient of variance < 1% (five repeated measures in twenty patients, data not shown). The enCORE® method has been validated against CT, the gold standard[7,25]. The short- and long-term coefficients of variation for our densitometer are 0.8% and 1.4%, respectively. The VAT short-term repeat measurement error CV is 9.8%[18].

Immunosuppression

All included patients used oral tacrolimus twice daily (Prograf®, Astellas Pharma US Inc.) with an initial dose of 0.04 mg/kg (0.05 mg/kg in high risk patients). High-risk patients were defined by panel reactive antibodies >20% and/or presence of donor-specific antibodies. Therapeutic drug monitoring was performed from first day after transplantation by measuring whole blood trough concentrations. The therapeutic ranges were 3-7 µg/L in standard risk

patients and 8-12 µg/L in high-risk patients. All patients also received mycophenolate mofetil 750 mg twice daily and a low-dose steroid protocol. The steroid protocol consisted of 250 mg intravenous methylprednisolone on the day of transplantation, followed by oral prednisolone (day 1-14: 20 mg/day, day 15-28: 15 mg/day, day 29-60: 10 mg/day, day 61-179: 7.5 mg/day and from day 180: 5 mg/day). Patients also received induction therapy with basiliximab (20 mg on day 0 and 4). High-risk patients received in addition to the above mentioned combination also induction therapy with intravenous human immune globulins and rituximab. As seen in the flowchart (Figure 1) five eligible patients used cyclosporine. These few patients were excluded since the diabetogenic effect may differ between cyclosporine and tacrolimus[26 27], and we wanted to obtain a most homogenous cohort.

Statistical analyses

All statistical analyses were conducted with SPSS version 22. Normal distribution was tested by visual inspection on histograms. Pearson Chi-Square and Jonckheere-Terpstra test were used as appropriate. CRP was log-transformated before used in linear regression. Descriptive data are presented as median with interquartile range (IQR). Univariable and multivariable linear regression analyses were used for assessment of association between fasting plasma glucose concentration and relevant variables. Variables used in multivariable linear regression were chosen based on p-value < 0.25 in univariable linear regression. Collinearity was tested by assessing correlations between variables and the tolerance value in the linear regression output. Cut-off point for collinearity was set to correlation > 0.6 and no tolerance value < 0.1.

RESULTS

Glucose metabolism improved during the first year after transplantation

Table 1 shows demographic data, kidney function and immunosuppressive medication for all 150 transplant recipients stratified for the glucose tolerance groups; NGT (n=81, 54%), IFG (n=38, 25%), IGT (n=17, 11%) and PTDM (n=14, 9%) at 1 year post transplant. The glucose metabolism was generally improved during the first year, see Figure 2. Patients with PTDM at 1 year post-transplant were significantly older (p=0.001), had a higher blood trough concentration of tacrolimus (P=0.007), and they had a marginally lower measured GFR(p=0.056)) compared to the other glucose tolerance groups. A significantly higher proportion of the patients with NGT were females (p=0.017).

Total fat and visceral fat differs between glucose tolerance groups at 1 year after transplantation

Table 2 shows the body composition and glucose metabolism indices for the patients with NGT, IFG, IGT and PTDM at 1 year post-transplant. BMI, total body mass, total body fat mass and VAT measures were significantly different among the different groups of glucose tolerance and the glucose measurements expressed differences between groups as expected. Plasma insulin values and HOMA-IR-index were higher with worsening glucose tolerance category, while HOMA-beta was lowest in those with PTDM.

Linear regression analyses

Table 3A shows univariable and multivariable linear regression analysis predicting fasting plasma glucose concentration. Recipient age, male gender, BMI, absolute amount VAT, $VAT_{\%totBFM}$, total body fat mass, CRP, fasting insulin, HOMA-IR-index and HOMA-beta were all independently associated with fasting plasma glucose concentration. Including all variables from the univariable linear regression with a $p<0.25$ (without multicollinearity) together into a multivariable model significantly explained 87.4% of the variability in fasting plasma glucose concentration ($p<0.001$). HOMA-IR-index, HOMA-beta, $VAT_{\%totBFM}$ and BMI were the only significant variables (table 3A). Using backward (p=0.10) and forward selection (p=0.05) procedure on the multivariable model, HOMA-IR-index (standardized beta 1.182, $p<0.001$), HOMA-beta (standardized beta -1.066, $p<0.001$), $VAT_{\%totBFM}$ (standardized beta 0.132, p=0.001) and BMI (standardized beta -0.098, p=0.015) were included variables in both procedures, explaining 87.2% of the variability in fasting plasma glucose concentration ($p<0.001$) (not shown in table). Removing BMI from the total model, 86.7% of the variability was explained, while removing $VAT_{\%totBFM}$ resulted in that 86.6% could be explained – the

partial R-square for BMI in the model was 0.007, while the partial R-square for $VAT_{\%totBFM}$ was 0.008.

Table 3B shows univariable and multivariable linear regression analysis predicting 2-hour plasma glucose concentration after an OGTT. Univariable linear regression yielded recipient age, living donor, absolute amount VAT, $VAT_{\%totBFM}$, blood trough concentration of tacrolimus and HOMA-index as significantly associated with 2-hour plasma glucose. Inclusion of variables from univariable linear regression with $p<0.3$ (without multicollinearity) in addition to BMI ($p=0.575$) in a multivariable linear regression model explained 31.9% of the variability in 2-hour plasma glucose, with only HOMA-IR index and HOMA-beta as significant variables (table 3B). Using backward ($p=0.10$) and forward selection procedure ($p=0.05$) on the multivariable model, HOMA-IR-index and HOMA-beta were the only significant variables with both selection procedures and $VAT_{\%totBFM}$ was the only other variable included with both selection procedures (but not significant), $p<0.001$ for the total models. Backward regression procedure explained 30.1% of the variability in 2-hour plasma glucose concentration (HOMA-IR-index, standardized beta 0.620, $p<0.001$; HOMA-beta, standardized beta -0.536, $p<0.001$; living donor, standardized beta -0.153, $p=0.076$; BMI standardized beta -0.174, $p=0.093$; $VAT_{\%totBFM}$ standardized beta 0.172, $p=0.094$), while forward selection procedure yielded a model explaining 27.3% of the variability in 2-hour plasma glucose concentration (HOMA-IR-index, standardized beta 0.569, $p<0.001$; HOMA-beta, standardized beta -0.496, $p<0.001$; blood trough concentration of tacrolimus, standardized beta 0.138, $p=0.138$; $VAT_{\%totBFM}$, standardized beta 0.128, $p=0.181$).

DISCUSSION

In the present study, we found a general improvement in glucose metabolism during the first year after transplantation and the incidence of PTDM was reduced from 13 to 9%. This reversibility of PTDM is in line with findings in previous studies[28,29]. VAT measures were strongly associated with hyperglycemia and PTDM at one year, also in line with our previous findingsin kidney transplant patients early after transplantation[7]. A novel finding in the present study was that measures of insulin resistance and insulin release both came out as predictors of plasma glucose concentrations. Previous studies have shown conflicting results, some favoring insulin release as the major mechanism behind PTDM[30], some favoring insulin resistance[31]. Actually insulin release and insulin resistance were both the absolute most robust covariates associated with plasma glucose but also $VAT_{\%totBFM}$ and BMI contributed significantly. In a multivariable linear model a total of 87.4% of the variability in fasting plasma glucose concentration could be explained by these variables. Moreover, VAT measures were stronger predictors of both fasting and 2-hour plasma glucose in univariable regressions, than BMI. BMI was significantly correlated only with fasting plasma glucose but not with 2-hour plasma glucose in our analysis. In multivariable linear regression predicting fasting plasma glucose $VAT_{\%totBFM}$ and BMI were approximately equally important. Neither $VAT_{\%totBFM}$ nor BMI were significant variables in a multivariable linear model forprediction of 2-hour plasma glucose concentration, but insulin resistance and beta cell function explained 31.9% of the variability.

One year after transplantation, the absolute amount VAT and $VAT_{\%totBFM}$ were different among the groups of glucose tolerance. The VAT measurements were lowest in patients with NGT, and highest in patients with PTDM with intermediate values in those with IFG and IGT. The same difference was found for insulin resistance and also for CRP, although differences in CRP were only borderline significant. Observations in a healthy Japanese cohort have also demonstrated an association between VAT and insulin resistance[32], and a higher state of systemic low-grade inflammation in individuals with higher amount of VAT and abdominal fat distribution makes highest values of CRP in patients with PTDM expectable[33].

BMI, total body mass and total body fat mass increased according to worsening glucose tolerance (higher values in those with IGT than those with IFG, and higher values in those with IFG than those with NGT), but actually patients with PTDM seemed to have lower or similar median values of BMI and total body fat as those with prediabetes (IFG and IGT).

A possible explanation for the high $VAT_{\%totBFM}$ in patients with PTDM may be hereditary factors contributing to obesity and distribution of fat.. Although it is incontestable that obesity results from energy intake exceeding energy expenditure, also genetics[34,35], hormone secretion (steroid hormones, growth hormones) and function of various feedback mechanisms modulate both the amount of total body fat and the distribution of fat[36]. Patients fulfilling criteria for PTDM at one year post-transplant might have agenetic predispositionand as suchsusceptible for storage of a higher $VAT_{\%totBFM}$. Use of corticosteroids is a known determinant for obesity and weight gain after transplantation[37], stimulating a more abdominal distribution of fat[38]. Patients with PTDM did not have a higher median daily dosage of prednisolone than the rest of the patients, but they had a higher through value of tacrolimus that is known to be diabetogenic[39]

According to the World Health Organization BMI 25-29.9 kg/m^2 represents overweight and BMI > 30 kg/m^2 represents obesity[40]. Our transplant program has set a limit of BMI < 30 kg/m^2 for kidney recipients due to risk of surgical complications. The BMI ranged from 17.6 to 39.1 kg/m^2 at one year posttransplant in the present cohort. This limits the assessability of BMI as a covariate, but on the other hand it reflects the true clinical situation. Anyhow, using BMI > 30 kg/m^2 as the definition of obesity also implicates that individuals with a high amount of lean mass (muscle) and low fat mass will be categorized as obese. This is not favorable when it is the distribution of fat and especially abdominal obesity that is the key correlate to the adverse effects of obesity and not BMI[41].

VAT seems to be representative of whole-body adipose tissue dysfunction. As such, accumulation of VAT is a consequence of limited storage of fat in peripheral subcutaneous adipose tissue, and this limited storage results in energy overflow, lipotoxicity and fatty infiltration in non-adipose tissue including visceral accumulation[15]. Decreasing VAT selectively through omentectomy has failed to improve glucose metabolism in obese patients[42]. It is therefore probably more important to reduce whole-body fat dysfunction than VAT by itself[17]. Obesity leads to a dysregulated secretion of adipokines with essential roles including regulation of appetite and satiety, distribution of fat, insulin sensitivity and insulin secretion. Prophylaxis and treatment of obesity are probably by far the best approach including stimulation to physical activity and reduced caloric intake. The importance of lifestyle measures for improvement of outcomes in kidney transplant patients is a topic of increasing interest. There are some evidence that active lifestyle modifications in these patients could improve glucose metabolism[43] and a randomized controlled study assessing

nutrition intervention and exercise has recently been launched[44]. In our clinic, all patients were offered free admittance to training facilities and generally exercise at least 3 times weekly during the first 8 weeks after transplantation, and they are encouraged to continue an active lifestyle after discharge from the hospital.

The findings in our study pinpoint the role of VAT in post transplant hyperglycemia and PTDM. The study demonstrates that VAT measurements is feasible in larger cohorts of patients and allow for future clinical studies on the mechanisms and hopefully treatment options of modifiable factors associated with PTDM. It should also be kept in mind that BMI and VAT changes over time are not necessarily mirrored.

Our study has some limitations. It was restricted to Caucasians and the results may not apply to other ethnical groups. The number of patients in each glucose tolerance group was limited. We performed OGTT only once, and not twice which is preferable to establish the diagnosis of diabetes[21]. Fewer patients had abnormal 2-hour plasma glucose than fasting plasma glucose, possible precluding significant associations in the multivariable linear regression predicting 2-hour plasma glucose. Furthermore, the present study is observational and we have only demonstrated associations and not causality.

In conclusion, we have demonstrated an improvement of glucose metabolism one year after transplantation and that VAT measurements are strongly associated with hyperglycemia one year after kidney transplantation. VAT along with measures of insulin resistance and release were the most robust markers of glucose metabolism at one year post-transplant.

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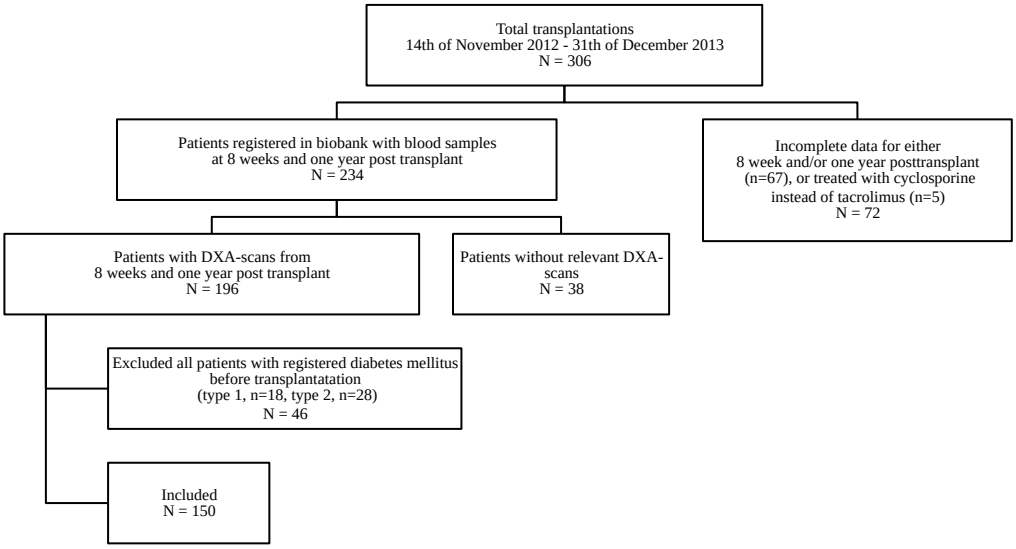
TABLES/FIGURE LEGENDS/FIGURES

	Title	Legend
Table 1	Demographic data and body composition at one year post-transplant	A: Pearson Chi-Square B: Jonckheere-Terpstra test for ordered alternatives
Table 2	Comparison of body composition and glucose metabolism between patients with different glucose tolerance at one year post-transplant	A: Pearson Chi-Square B: Jonckheere-Terpstra test for ordered alternatives
Table 3A	Univariable and multivariable linear regression of determinants of fasting plasma glucose concentration at one year post-transplant	Unstandardized coefficients refer to how many units the dependent variable changes per unit change in an independent variable. Standardized coefficients refer to how many standard deviations the dependent variable changes per standard deviation change in the independent variable, and this shows which independent variable has a greater effect on the dependent variables
Table 3B	Univariable and multivariable linear regression of determinants of 2-hour glucose concentration at one year post-transplant	Unstandardized coefficients refer to how many units the dependent variable changes per unit change in an independent variable. Standardized coefficients refer to how many standard deviations the dependent variable changes per standard deviation change in the independent variable, and this shows which independent variable has a greater effect on the dependent variables
Figure 1	Flowchart of patients included in the analysis	Only data from one year post-transplant is presented in this article, but the selection of patients was based on patients with registered data from both 8-weeks and 1 year post transplant.
Figure 2		Change in glucose tolerance group from baseline (measured in a stable phase 8-10 weeks after transplantation) to follow-up 1 year post transplant.

Figure 1: Flowchart of patients included in the analysis



Only data from one year post-transplant is presented in this article, but the selection of patients was based on those with registered data from both 8-weeks and 1 year post transplant.

Figure 2:

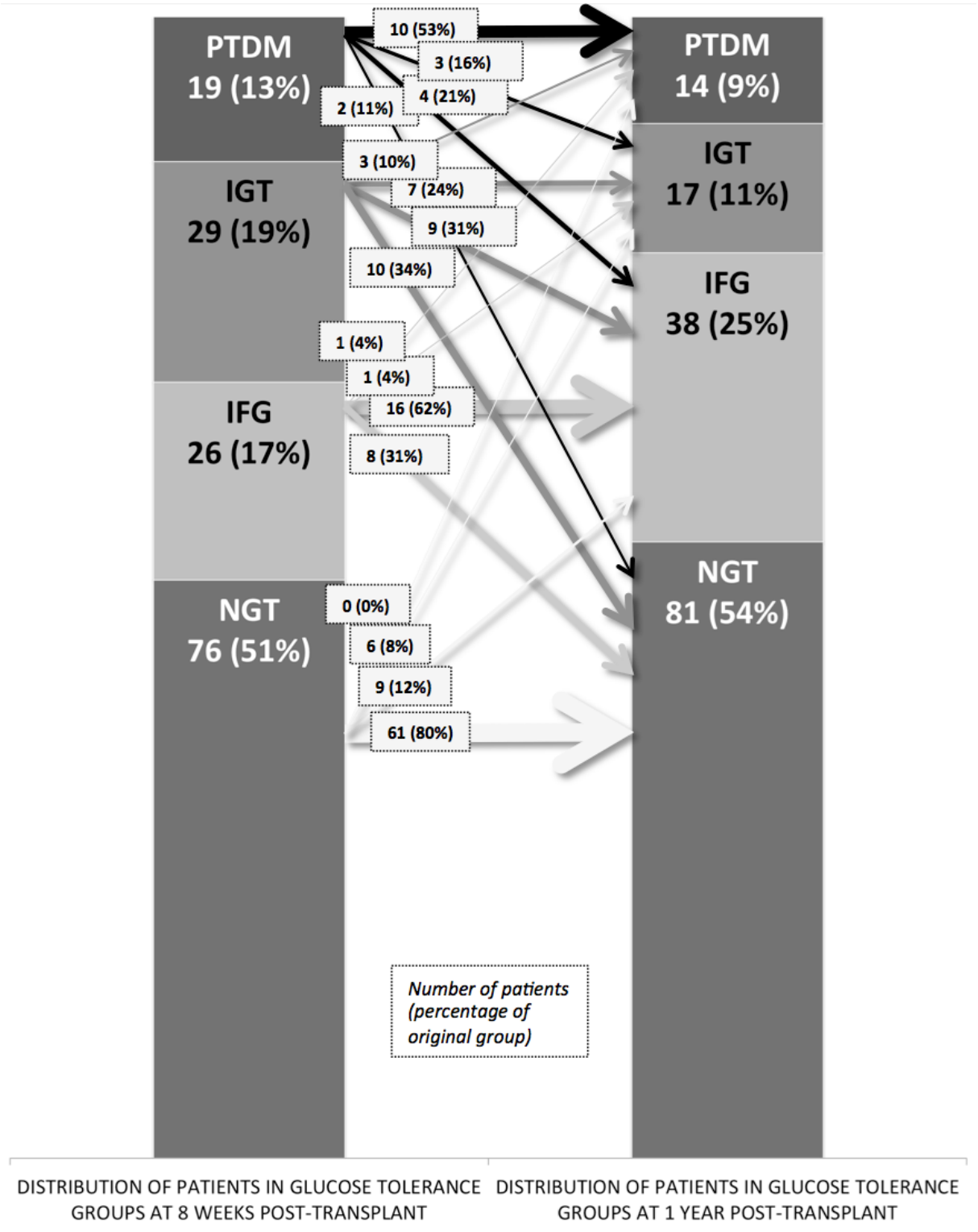


Table 1: Demographic data

	All patients (n=150)	Normal glucose tolerance (n=81)	Impaired fasting glucose (n=38)	Impaired glucose tolerance (n=17)	Post transplant diabetes mellitus (n=14)	P-value[D]
Age at time of transplant, median (IQR)	56.6 (44.1-65.4)	48.2 (41.7-60.5)	61.0 (48.1-68.8)	63.8 (54.1-69.9)	64.0 (53.9-69.6)	<0.001[B]
Male gender, n (%)	102 (68)	46 (57)	31 (82)	14 (82)	11 (79)	0.017[A]
First transplant, n (%)	130 (87)	66 (82)	35 (92)	15 (88)	14 (100)	0.168[A]
Preemptive transplantation, n (%)	47 (31)	29 (36)	14 (37)	1 (6)	3 (21)	0.070[A]
Time in dialysis[E] , months, median (IQR)	15 (7-25)	16 (4-28)	11 (4-23)	17 (10-23)	20 (13-33)	0.389[B]
Living donor, n (%)	43 (29)	25 (31)	12 (32)	4 (24)	2 (14)	0.576[A]
Acute rejection, n (%)	22 (15)	8 (10)	8 (21)	5 (29)	1 (7)	0.097[A]
Creatinine (µmol/L) at one year post-transplant, median (IQR)	105 (90-134)	98 (83-116)	108 (95-137)	137 (100-167)	107 (85-138)	0.003[B]
Measured GFR[C] at one year post-transplant, median (IQR)	56 (49-69)	58 (53-72)	56 (44-68)	55 (47-66)	52 (41-64)	0.056[B]
Tacrolimus C_0 at one year post-transplant, µg/L	5.8 (4.8-6.8)	5.5 (4.4-6.6)	6.2 (5.2-6.8)	6.0 (5.1-7.5)	6.8 (5.6-7.6)	0.007*[B]
Prednisolone dose at one year post-transplant, mg/day	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	5 (5-5)	0.986[B]

A: Pearson Chi-Square
B: Jonckheere-Terpstra test for ordered alternatives
C: GFR was measured based on clearance of a filtration marker and is given in mL/min/1.73m^2.
D: Comparison between glucose tolerance groups
E: for those without preemptive transplantation, (hemodialysis and peritoneal dialysis)

Table 2: Comparison of body composition and glucose metabolism between patients in different glucose tolerance categories at 1 year post-transplant

	All patients (n=150)	Normal glucose tolerance (n=81)	Impaired fasting glucose (n=38)	Impaired glucose tolerance (n=17)	Post transplant diabetes mellitus (n=14)	P-value
Body composition						
Body mass index (kg/m^2), median (IQR)	24.7 (22.6-28.2)	24.0 (22.3-26.6)	25.6 (23.2-30.4)	28.2 (24.5-29.4)	24.8 (23.6-26.6)	0.005*
Total body mass (kg), median (IQR)	76.9 (65.7-87.0)	73.0 (62.4-84.2)	81.2 (70.5-95.2)	81.4 (74.6-90.1)	74.2 (64.1-81.6)	0.039*
Total body fat (kg), median (IQR)	25.0 (17.9-30.6)	21.9 (16.5-29.4)	25.6 (18.6-31.6)	28.1 (25.4-35.2)	25.9 (18.0-28.2)	0.021*
Visceral fat (kg), median (IQR)	1.0 (0.5-1.8)	0.9 (0.3-1.4)	1.1 (0.8-2.7)	1.5 (1.2-2.2)	1.5 (0.7-2.1)	<0.001*
Percentage visceral fat of total body fat, median (IQR)	4.3 (2.7-6.3)	3.8 (1.6-5.0)	5.7 (3.0-7.3)	5.8 (4.4-7.5)	6.6 (4.6-7.8)	<0.001*
Glucose metabolism						
Fasting glucose (mmol/L), median (IQR)	5.4 (5.0-5.9)	5.1 (4.7-5.3)	5.9 (5.7-6.2)	5.9 (5.5-6.3)	7.2 (6.7-7.5)	<0.001*
2 hour plasma glucose (mmol/L), median (IQR)	6.1 (5.2-7.1)	5.4 (4.7-6.2)	6.4 (5.4-6.9)	8.3 (8.1-9.0)	13.3 (10.6-15.2)	<0.001*
HbA1c (% glycated hemoglobin), median (IQR)	5.8 (5.4-6.1)	5.6 (5.3-5.8)	5.9 (5.6-6.3)	6.0 (5.8-6.3)	6.6 (6.3-7.4)	<0.001*
Fasting insulin (pmol/L) , median (IQR)	2.3 (1.6-3.1)	2.1 (1.4-2.9)	2.5 (2.0-3.1)	2.6 (1.8-3.4)	2.7 (2.1-3.6)	0.006*
Fasting proinsulin (pmol/L) , median (IQR)	1.9 (0.8-3.5)	2.1 (0.9-3.7)	1.8 (0.5-3.5)	1.5 (0.6-2.4)	3.3 (1.1-6.8)	0.708
Fasting proinsulin:fasting insulin-ratio (%), median (IQR)	0.7 (0.4-1.6)	0.8 (0.4-1.8)	0.7 (0.3-1.3)	0.6 (0.3-1.0)	1.0 (0.4-1.9)	0.265
HOMA-IR-index, median (IQR)	3.8 (2.5-5.4)	3.2 (2.1-4.6)	4.5 (3.5-5.6)	4.6 (2.7-6.5)	6.1 (4.8-8.4)	<0.001*
HOMA-beta, median (IQR)	167 (117-231)	198 (138-271)	142 (105-178)	167 (117-215)	101 (73-145)	<0.001*
Systemic inflammation						
C-reactive protein (mg/l), median (IQR)	1.9 (0.7-4.9)	1.4 (0.7-3.9)	1.9 (0.5-4.8)	2.3 (1.3-5.9)	2.9 (1.4-7.2)	0.085

A: Pearson Chi-Square

B: Jonckheere-Terpstra test for ordered alternatives

Table 3A: Univariable and multivariable linear regression of determinants of fasting plasma glucose concentration at 1 year post-transplant

	Univariable linear regression				Multivariable linear regression		
	R square	Unstandarized beta	Standarized beta	P-value	Standarized beta	P-value	R2
Recipient age	0.065	0.013	0.255	0.002*	-0.043	0.283	0.874
Male gender	0.056	0.361	0.238	0.004*	0.003	0.936	
Living donor	0.009	-0.148	-0.095	0.258			
Body mass index (kg/m2)	0.044	0.034	0.209	0.012*	-0.104	0.015*	
Visceral fat (gram)	0.140	0.000	0.374	<0.001*			
Total body fat (gram)	0.034	0.000	0.185	0.026*			
% Visceral fat of total body fat mass	0.183	12.294	0.428	<0.001*	0.152	0.007*	
Prednisolone, daily dosage, mg	0.002	0.028	0.041	0.641			
Tacrolimus (whole blood trough concentration), µg/L	0.010	0.044	0.099	0.242			
CRP	0.031	0.259	0.127	0.043*	-0.015	0.668	
Measured GFR	0.015	-0.006	-0.124	0.189			
Fasting insulin	0.072	0.019	0.269	0.002*			
Proinsulin	0.000	0.004	0.018	0.842			
HOMA-IR-index	0.208	0.130	0.456	<0.001*	1.192	<0.001*	
HOMA-beta	0.078	-0.002	-0.279	0.001*	-1.077	<0.001*	

Unstandardized coefficients refer to how many units the dependent variable changes per unit change in an independent variable. Standardized coefficients refer to how many standard deviations the dependent variable changes per standard deviation change in the independent variable, and this shows which independent variable has a greater effect on the dependent variables

Table 3B: Univariable and multivariable linear regression of determinants of 2 hour plasma glucose concentration after an OGTT at 1 year post-transplant

	Univariable linear regression				Multivariable linear regression		
	R square	Unstandarized beta	Standarized beta	P-value	Standarized beta	P-value	R2
Recipient age	0.072	0.045	0.268	0.001*	0.037	0.725	0.319
Male gender	0.013	0.545	0.398	0.172	-0.056	0.602	
Living donor	0.037	-0.950	-0.192	0.020*	-0.124	0.153	
Body mass index (kg/m2)	0.002	0.024	0.047	0.575	-0.169	0.110	
Visceral fat (gram)	0.052	0.000	0.228	0.006*			
Total body fat (gram)	0.010	0.000	0.101	0.225			
% Visceral fat of total body fat mass	0.080	25.582	0.283	0.001*	0.189	0.179	
Prednisolone, daily dosage, mg	0.000	0.040	0.019	0.831			
Tacrolimus (whole blood trough concentration), µg/L	0.031	0.249	0.176	0.036*	0.106	0.250	
CRP	0.004	0.025	0.059	0.501			
Measured GFR	0.020	-0.022	-0.140	0.137	-0.048	0.591	
Fasting insulin	0.017	0.030	0.132	0.125			
Proinsulin	0.000	0.008	0.012	0.892			
HOMA-IR-index	0.059	0.217	0.242	0.005*	0.604	<0.001*	
HOMA-beta	0.023	-0.003	-0.151	0.078*	-0.492	<0.001*	

Unstandardized coefficients refer to how many units the dependent variable changes per unit change in an independent variable. Standardized coefficients refer to how many standard deviations the dependent variable changes per standard deviation change in the independent variable, and this shows which independent variable has a greater effect on the dependent variables