Supplementary material to

Glucose control is associated with patient and graft survival in diabetic patients after renal transplantation
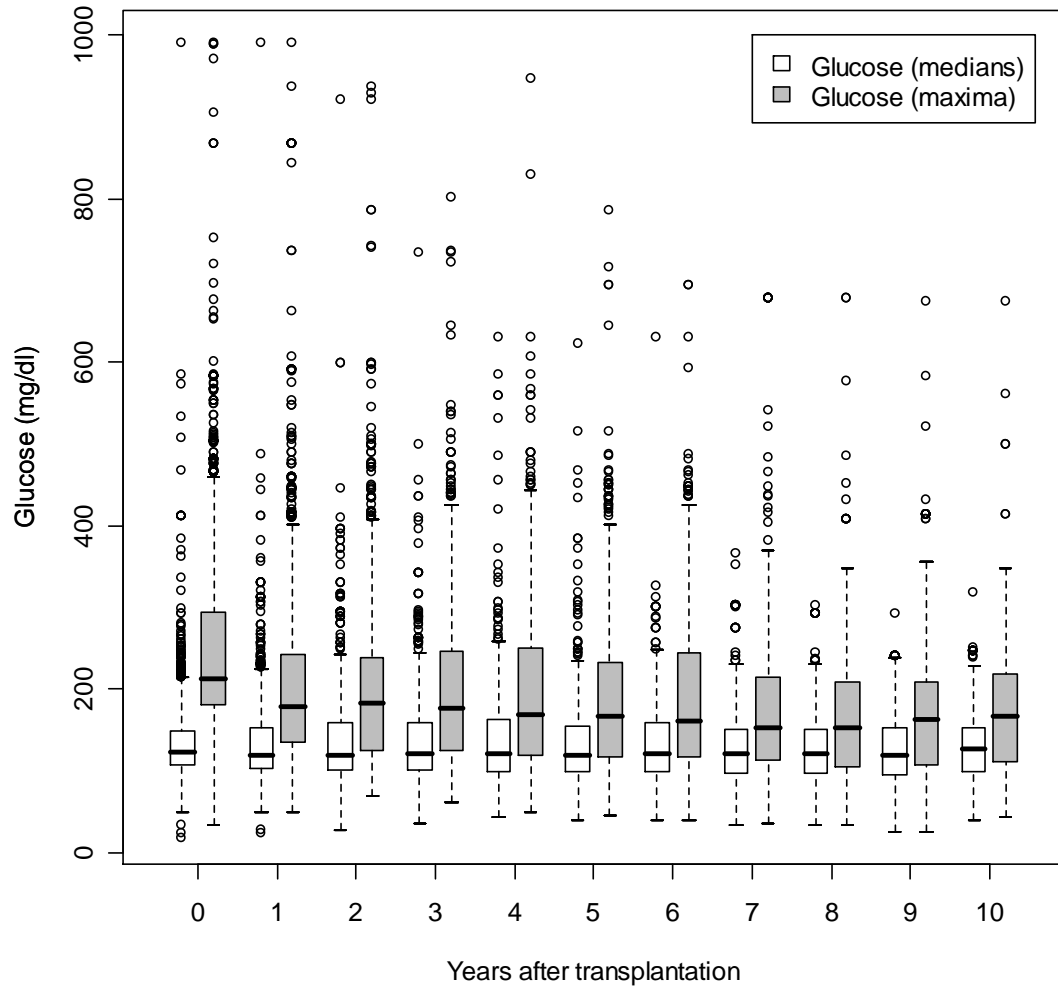
by

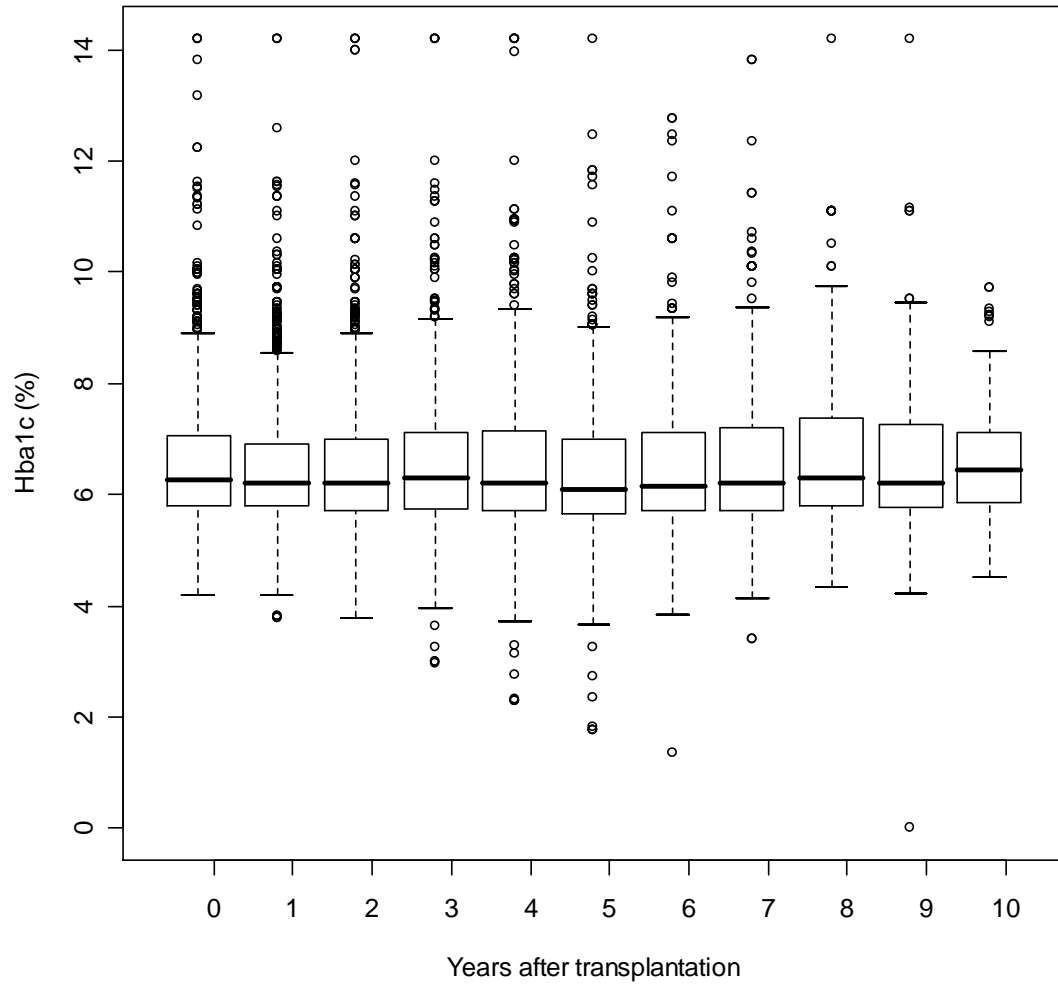Franz Wiesbauer *et al*

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1.   Glucose (medians/maxima per patient and year) (truncated at 1000 units)

2.   Hba1c (median per patient and year)

3.   Cox-regression: complete-cases-only analyses

Analysis is adjusted for:
Number of antihypertensive drugs, cholesterol level, type of IS, year of TPL, MAP, donor age

outcome	Parameter	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > ChiSq
actual graft survival	hba1c_q1	0.76	0.50	1.17	0.2158
actual graft survival	hba1c_q2	0.92	0.60	1.41	0.6946
actual graft survival	hba1c_q3	0.84	0.57	1.25	0.3894
actual graft survival	Trend	1.12	0.95	1.30	0.1678
functional graft surv	hba1c_q1	0.82	0.47	1.41	0.4722
functional graft surv	hba1c_q2	0.96	0.55	1.68	0.8950
functional graft surv	hba1c_q3	0.82	0.49	1.36	0.4396
functional graft surv	Trend	1.03	0.84	1.26	0.7901
patient survival	hba1c_q1	0.69	0.34	1.38	0.2939
patient survival	hba1c_q2	0.84	0.43	1.65	0.6157
patient survival	hba1c_q3	0.86	0.47	1.58	0.6318
patient survival	Trend	1.23	0.97	1.58	0.0917

outcome	Parameter	Hazard Ratio	95% Lower Confidence Limit for Hazard Ratio	95% Upper Confidence Limit for Hazard Ratio	Pr > ChiSq
actual graft survival	glukose_max _q1	1.29	0.85	1.97	0.2345
actual graft survival	glukose_max _q2	1.48	0.99	2.21	0.0550
actual graft survival	glukose_max _q3	1.48	0.99	2.22	0.0572
actual graft survival	Trend	1.19	1.05	1.35	0.0074
functional graft surv	glukose_max _q1	1.16	0.67	2.00	0.6056
functional graft surv	glukose_max _q2	1.22	0.71	2.09	0.4666
functional graft surv	glukose_max _q3	1.16	0.69	1.96	0.5799
functional graft surv	Trend	1.04	0.89	1.23	0.6171
patient survival	glukose_max _q1	1.57	0.81	3.05	0.1804
patient survival	glukose_max _q2	1.96	1.06	3.63	0.0313
patient survival	glukose_max _q3	2.07	1.09	3.91	0.0258
patient survival	Trend	1.46	1.19	1.80	0.0004

Complete-case-only analysis for Treatment comparison

Crude hazard ratios:

outcome	label	HazardRatio	HRLowerCL	HRUpperCL	ProbChiSq
Patient survival	Insulin vs. diet	1.83	1.09	3.08	0.0224
Patient survival	Insulin vs. oral	2.74	1.30	5.76	0.0078
Functional graft survival	Insulin vs. diet	0.93	0.53	1.64	0.8067
Functional graft survival	Insulin vs. oral	4.11	1.38	12.28	0.0113
Actual graft survival	Insulin vs. diet	1.31	0.90	1.91	0.1600
Actual graft survival	Insulin vs. oral	3.16	1.71	5.83	0.0002

Adjusted hazard ratios (multivariable Cox model; adjusted for number of antihypertensive drugs, peripheral vascular disease, cholesterol level, maximal glucose level, type of immunosuppression, year of transplantation, diabetes duration, donor age; 65 deaths, 65 graft losses):

outcome	label	HazardRatio	HRLowerCL	HRUpperCL	probt
Patient survival	Insulin vs. diet	1.04	0.47	2.30	0.926
Patient survival	Insulin vs. oral	1.50	0.61	3.71	0.382
Functional graft survival	Insulin vs. diet	1.08	0.38	3.05	0.888
Functional graft survival	Insulin vs. oral	3.60	1.05	12.37	0.042
Actual graft survival	Insulin vs. diet	1.03	0.55	1.91	0.933
Actual graft survival	Insulin vs. oral	2.03	0.96	4.26	0.063

Adjusted hazard ratio estimates from marginal structural Cox models; adjusted for number of antihypertensive drugs, peripheral vascular disease, cholesterol level, maximal glucose level, type of immunosuppression, year of transplantation, diabetes duration, donor age; 65 deaths, 65 graft losses):

outcome	label	HazardRatio	HRLowerCL	HRUpperCL	probt
Patient survival	Insulin vs. diet	1.23	0.55	2.75	0.618
Patient survival	Insulin vs. oral	1.99	0.79	4.98	0.142
Functional graft survival	Insulin vs. diet	0.91	0.28	2.94	0.878
Functional graft survival	Insulin vs. oral	3.71	0.91	15.10	0.067
Actual graft survival	Insulin vs. diet	1.05	0.54	2.05	0.881
Actual graft survival	Insulin vs. oral	2.36	1.08	5.15	0.031

4. Crude hazard ratios for treatment comparison

outcome	label	hazardratio	hrlowercl	hruppercl	Probt
Patient survival	Insulin vs. diet	2.54	1.78	3.62	<.001
Patient survival	Insulin vs. oral	2.67	1.60	4.48	<.001
Functional graft survival	Insulin vs. diet	0.90	0.61	1.32	0.581
Functional graft survival	Insulin vs. oral	1.80	1.00	3.22	0.049
Actual graft survival	Insulin vs. diet	1.52	1.18	1.95	0.001
Actual graft survival	Insulin vs. oral	2.27	1.55	3.32	<.001

5.   Adjusted hazard ratios for treatment comparison

outcome	label	hazardratio	hrlowercl	hruppercl	Probt
Patient survival	Insulin vs. diet	1.66	0.93	2.96	0.087
Patient survival	Insulin vs. oral	2.00	1.08	3.68	0.026
Functional graft survival	Insulin vs. diet	1.30	0.64	2.63	0.463
Functional graft survival	Insulin vs. oral	1.48	0.69	3.17	0.315
Actual graft survival	Insulin vs. diet	1.37	0.91	2.07	0.132
Actual graft survival	Insulin vs. oral	1.73	1.08	2.75	0.022

Analysis is adjusted by:
Number of antihypertensive drugs
Cerebrovascular disease
Peripheral vascular disease
Cholesterol
Hba1c
Serum glucose (maximum per year)
Type of IS (4 groups)
Use of calcinurein inhibitors
MAP
Year of TPL
Cold ischemia time
Years of diabetes before TPL
Age at TPL
Donor age
Sum of HLA mismatches

6. **Nonlinear estimation of adjusted association of glucose control and (a) mortality, (b) functional graft survival, (c) actual graft survival.**

Nonlinear estimation was performed using restricted cubic splines with knots placed at the 5th, 35th, 65th and 95th percentiles of the empirical distribution of each parameter (median glucose per year, HbA1c, maximal glucose per year).
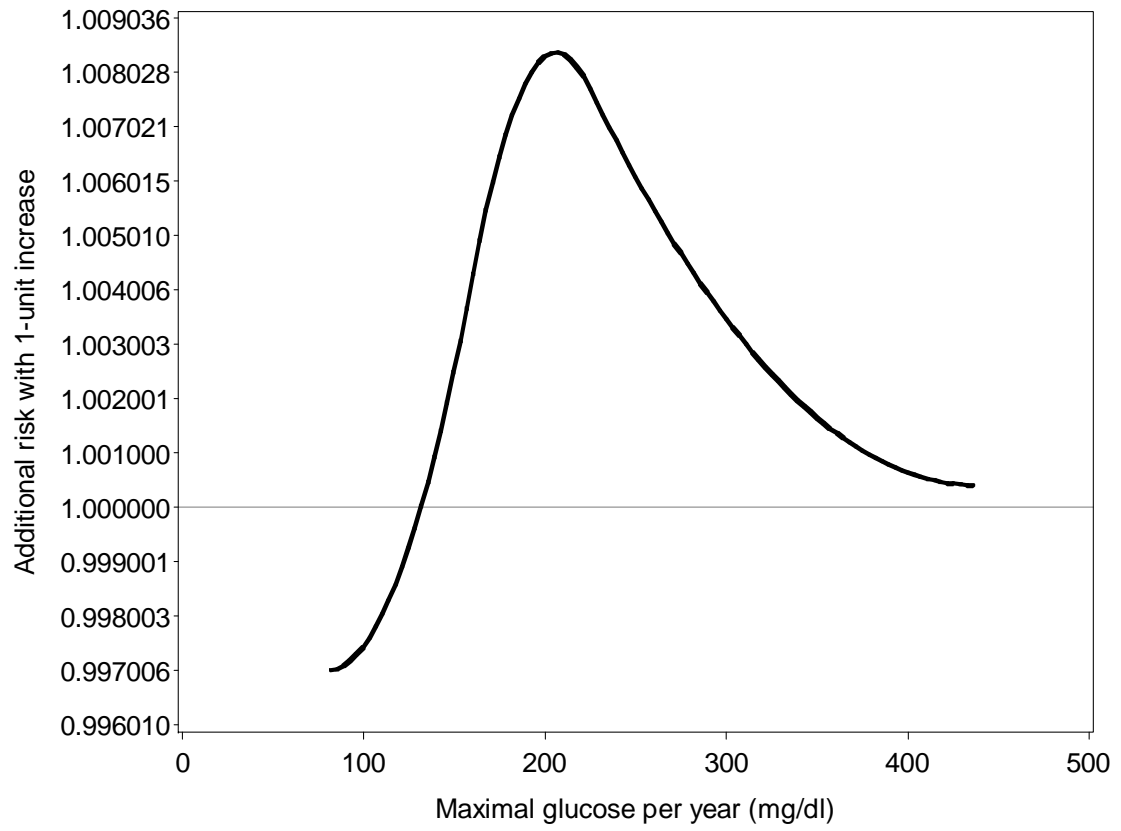
(a) patient survival
Neither mean glucose levels (mg/dl) nor mean HbA1c (%) predicted mortality. Solid line indicate the HR, dashed lines the 95%CI.
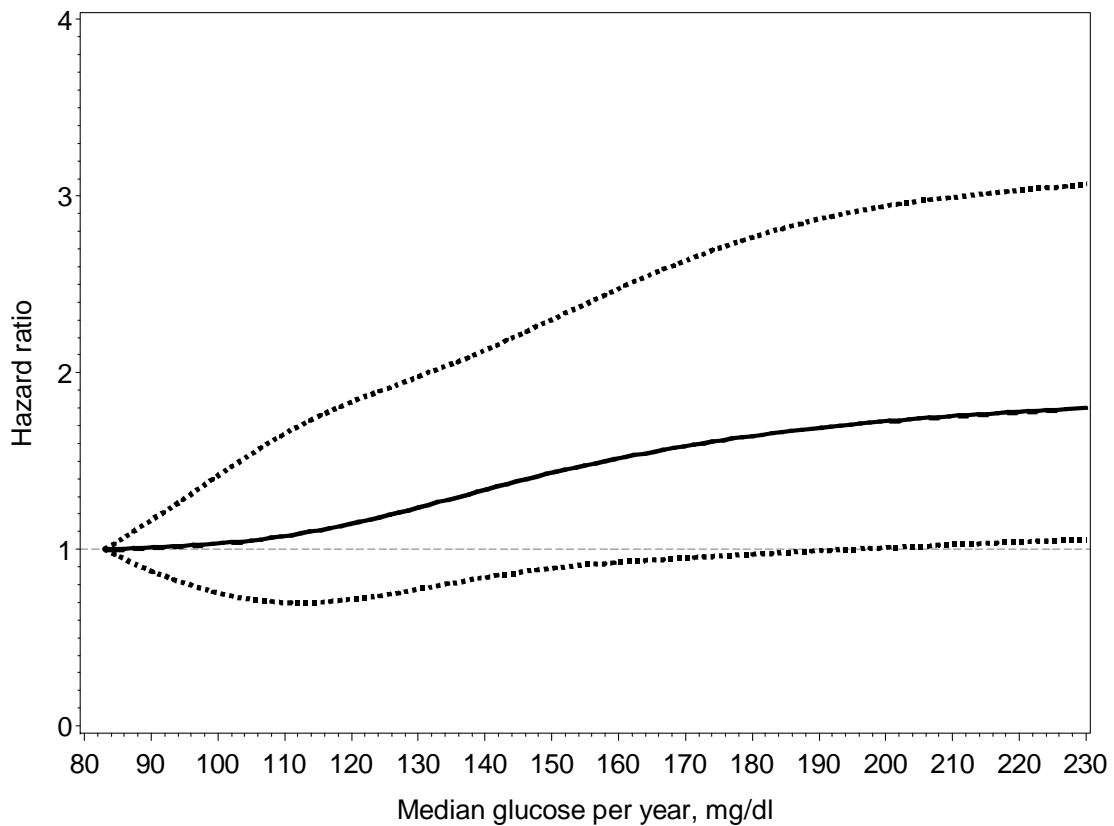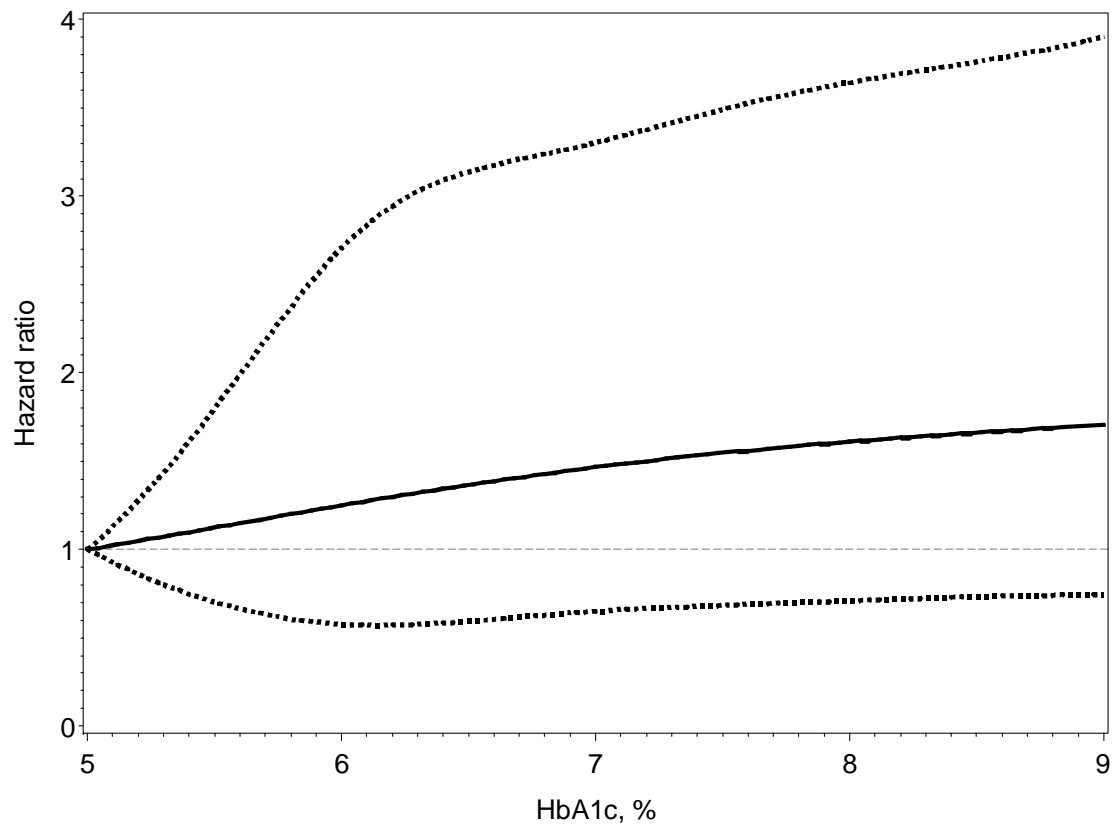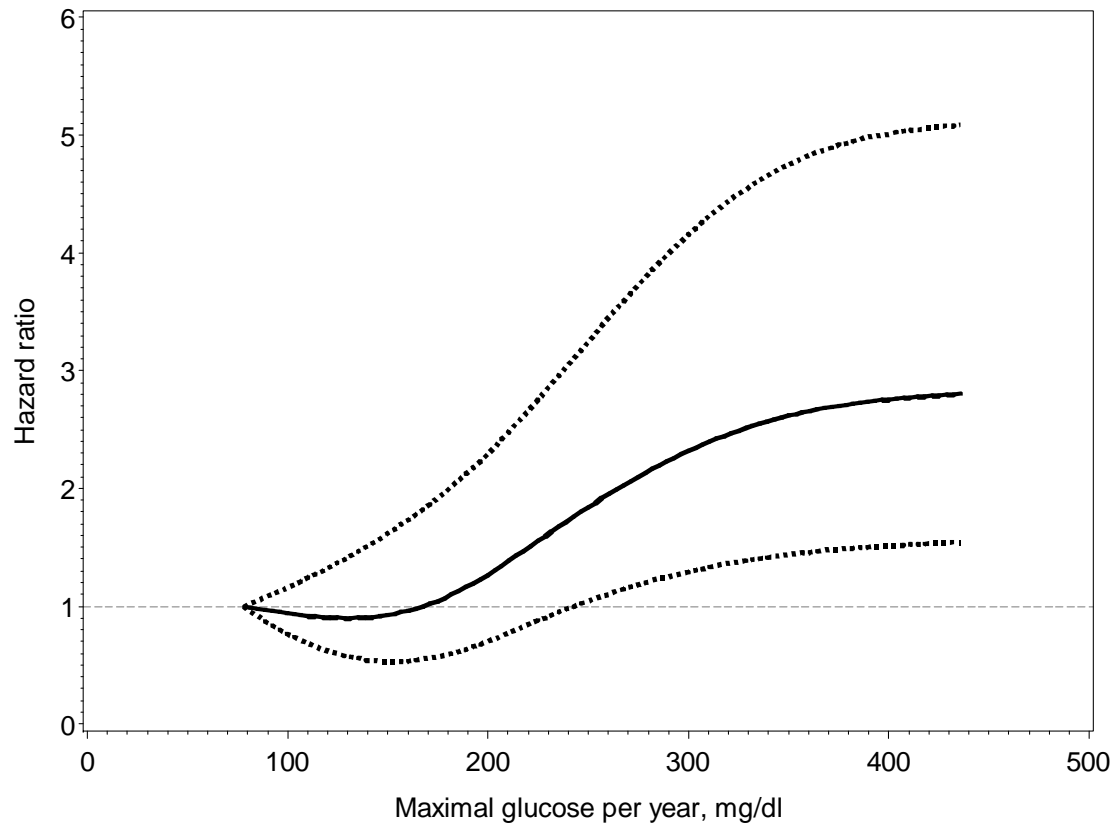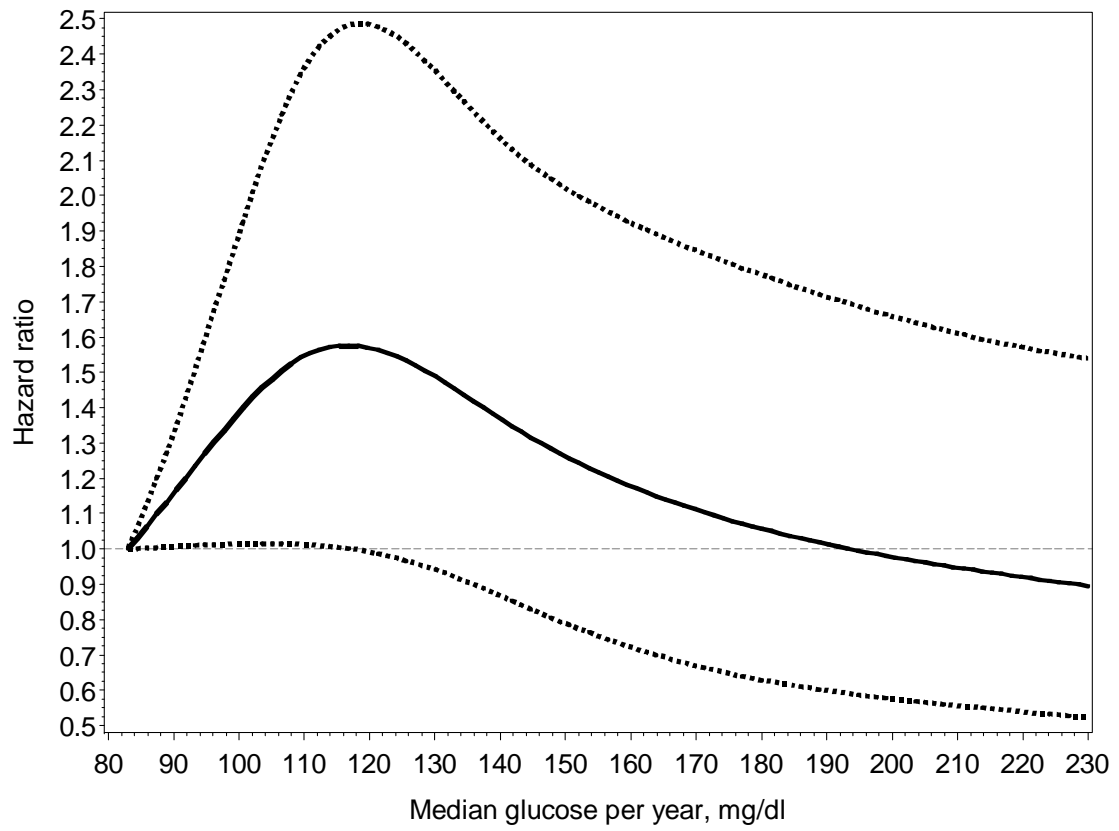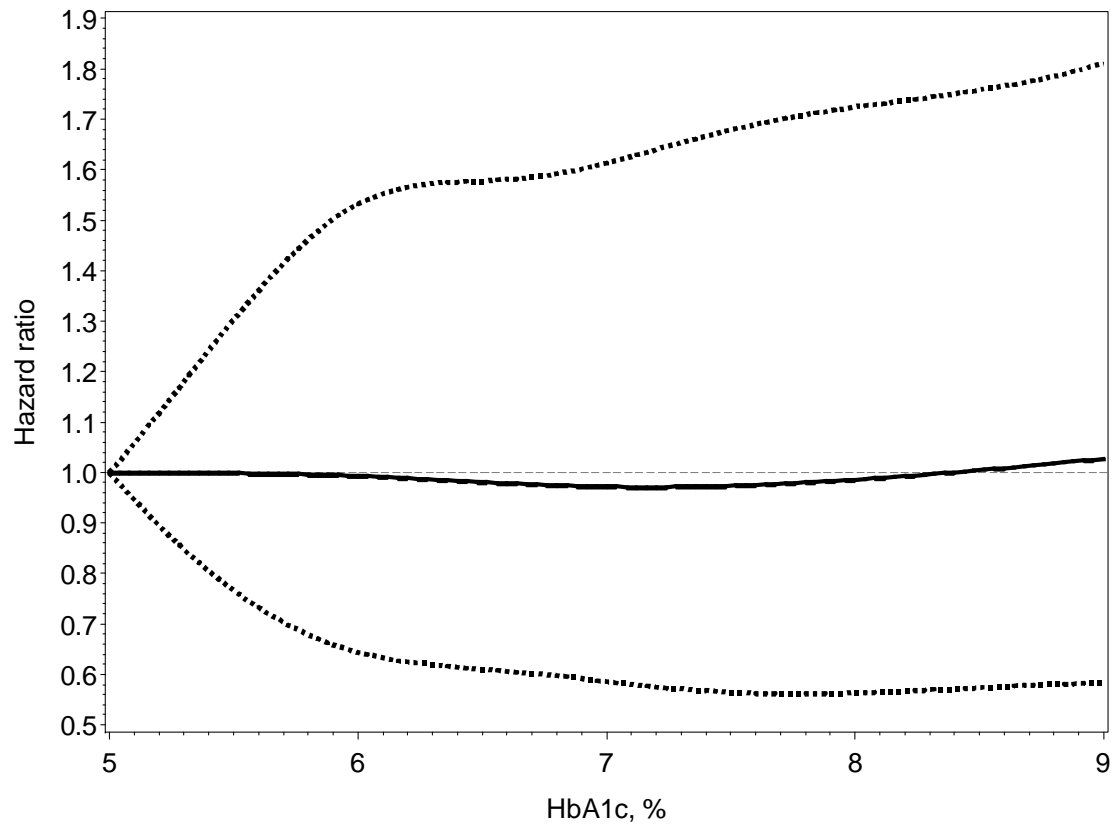
The slope of this curve reaches a maximum at 207, i. e. at 207 there is maximal additional risk due to increase of maximal glucose by 1 unit:

Y-axis: Additional risk with 1-unit increase

Y-axis values (top to bottom): 1.009036, 1.008028, 1.007021, 1.006015, 1.005010, 1.004006, 1.003003, 1.002001, 1.001000, 1.000000, 0.999001, 0.998003, 0.997006, 0.996010

X-axis: Maximal glucose per year (mg/dl)

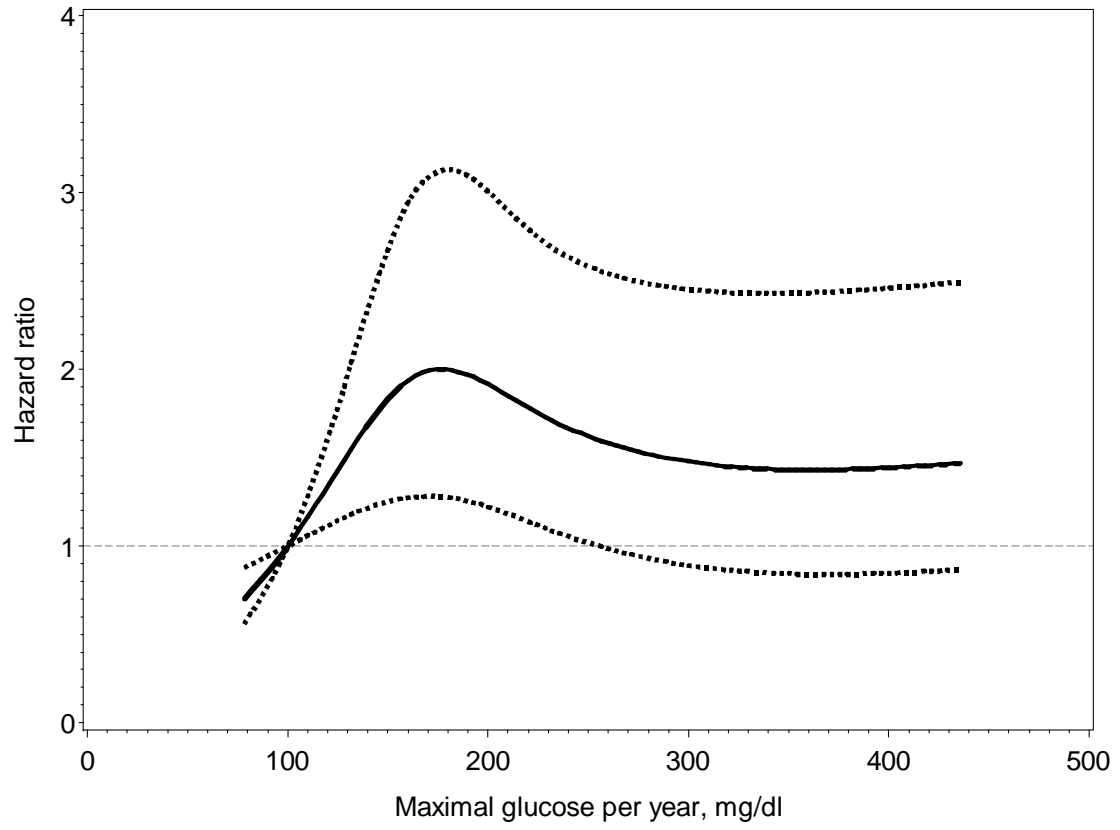X-axis values: 0, 100, 200, 300, 400, 500
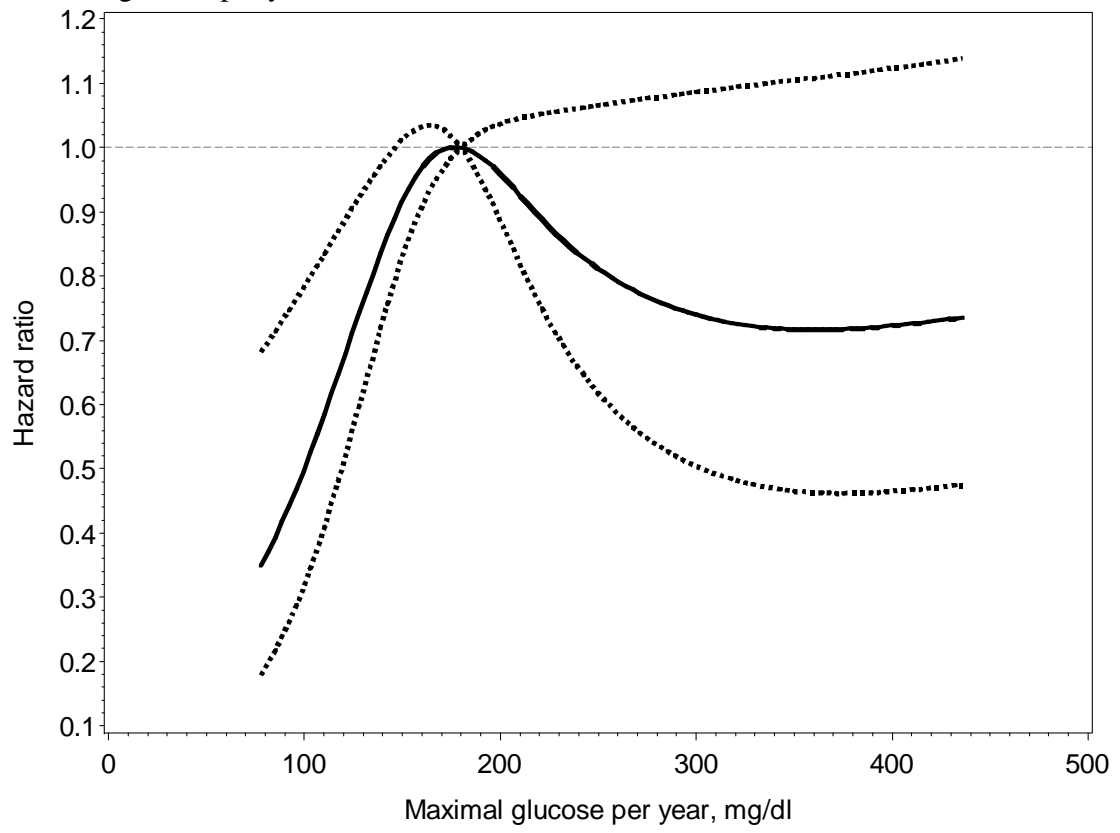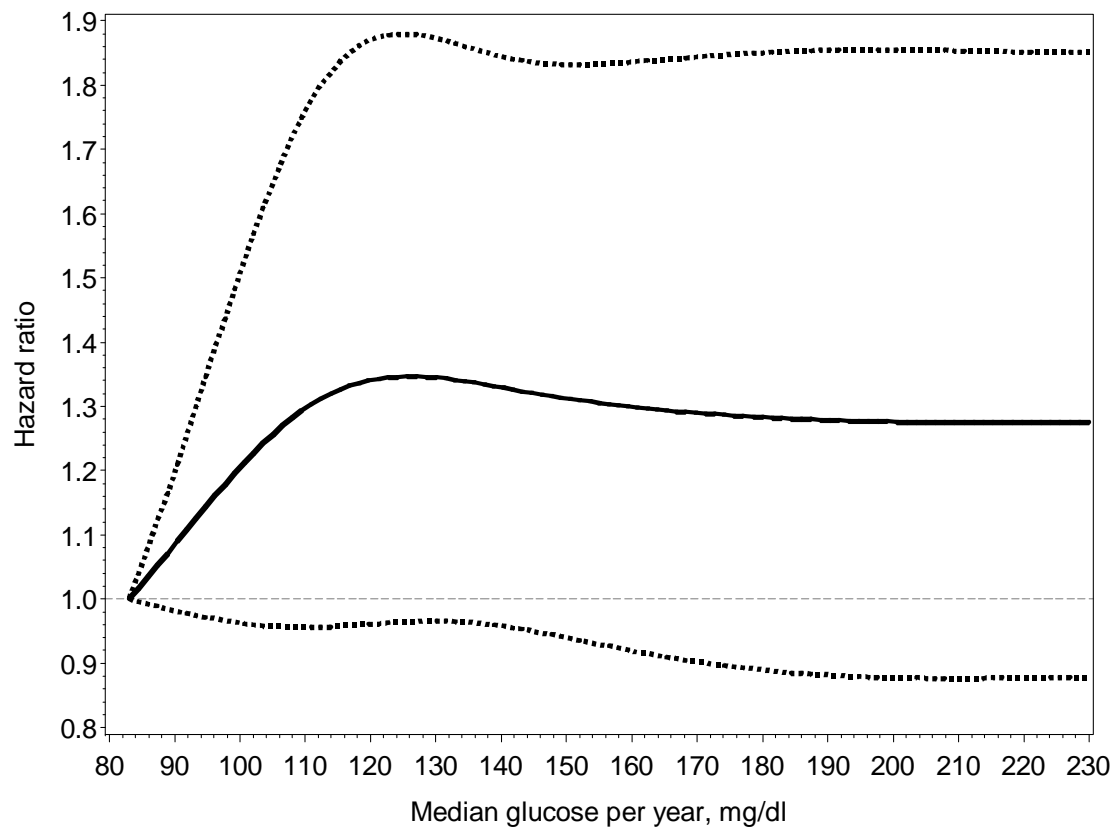
(b) Functional graft survival
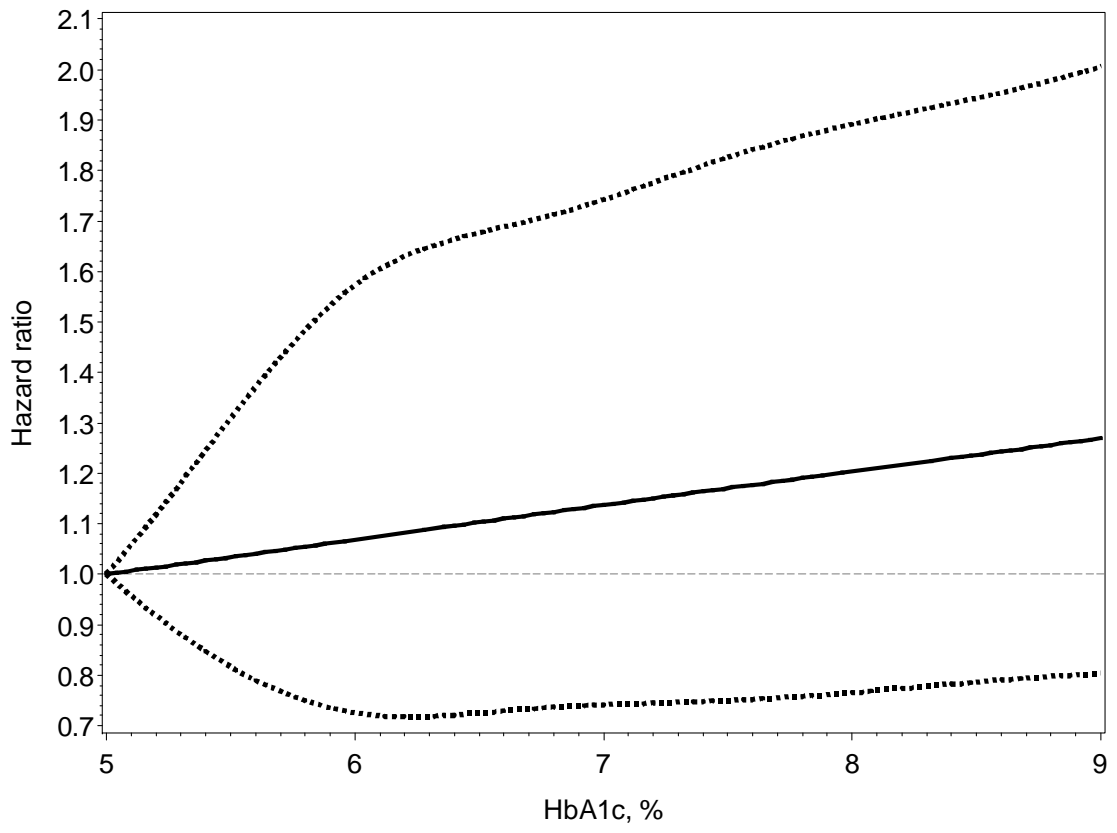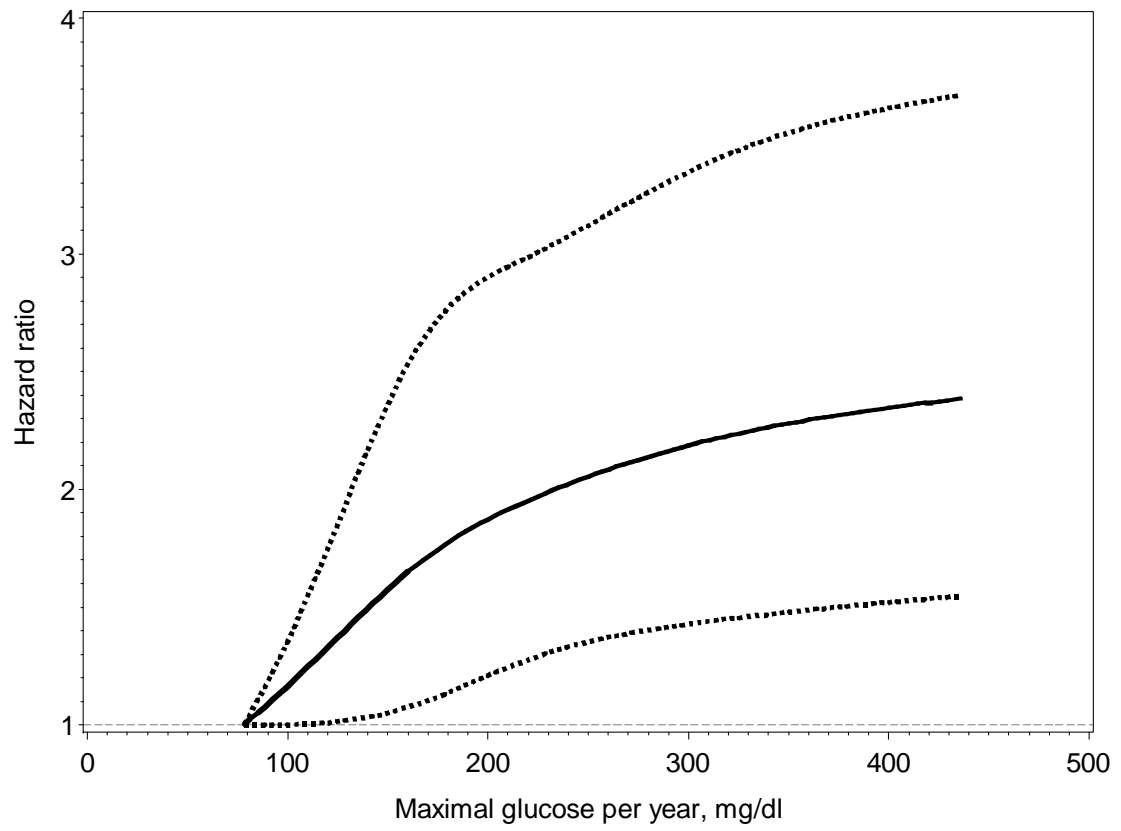
Maximal glucose per years, reference value 100:

Maximal glucose per years, reference value 180:

(c) Actual graft survival

7.   Assessment of model assumptions

We assessed violation of model assumptions in the following Cox regression models:
Model 1: Patient survival, maximal glucose
Model 2: Functional graft survival, maximal glucose
Model 3: Actual graft survival, maximal grlucose
Model 4: Patient survival, HbA1c
Model 5: Functional graft survival, HbA1c
Model 6: Actual graft survival, HbA1c

In Models 1-6, nonlinear (quadratic and cubic) effects for the continuous variables cholesterol level, donor age and MAP were included. In Models 1, 3, 4 and 6 interactions of log survival time with number of antihypertensive drugs and year of transplantation were included – these interactions were significant at a false discovery rate of 5%. Likewise, in models 2 and 5, interactions of log survival time with donor age were included because of their significance at an FDR of 5%. The resulting adjusted hazard ratio for quartiles of maximal glucose or HbA1c were slightly more pronounced than in the original analysis as reported in the paper.

Re-analysis for maximal glucose:

outcome	Quartile	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
actual graft survival	2 vs. 1	1.28	0.87	1.87	0.211
actual graft survival	3 vs. 1	1.37	0.96	1.95	0.083
actual graft survival	4 vs. 1	1.73	1.24	2.42	0.001
actual graft survival	Trend	1.19	1.07	1.33	0.002
functional graft surv	2 vs. 1	1.17	0.72	1.90	0.534
functional graft surv	3 vs. 1	0.99	0.60	1.63	0.976
functional graft surv	4 vs. 1	1.14	0.73	1.77	0.570
functional graft surv	Trend	1.03	0.90	1.19	0.649
patient survival	2 vs. 1	1.57	0.86	2.86	0.143
patient survival	3 vs. 1	1.99	1.20	3.31	0.008
patient survival	4 vs. 1	2.79	1.70	4.57	<.001
patient survival	Trend	1.40	1.19	1.64	<.001

Re-analysis for HbA1c:

| outcome	Quartile	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
actual graft survival	2 vs. 1	1.17	0.73	1.88	0.501
actual graft survival	3 vs. 1	1.26	0.78	2.06	0.331
actual graft survival	4 vs. 1	1.49	1.05	2.10	0.024
actual graft survival	Trend	1.13	1.02	1.27	0.024
functional graft surv	2 vs. 1	1.04	0.58	1.89	0.889
functional graft surv	3 vs. 1	1.33	0.72	2.45	0.356
functional graft surv	4 vs. 1	1.06	0.62	1.80	0.834
functional graft surv	Trend	1.03	0.87	1.22	0.705
patient survival	2 vs. 1	1.33	0.68	2.60	0.392
patient survival	3 vs. 1	1.26	0.65	2.47	0.485
patient survival	4 vs. 1	2.18	1.25	3.83	0.007
patient survival	Trend	1.28	1.05	1.56	0.017

8. Results including actual graft survival

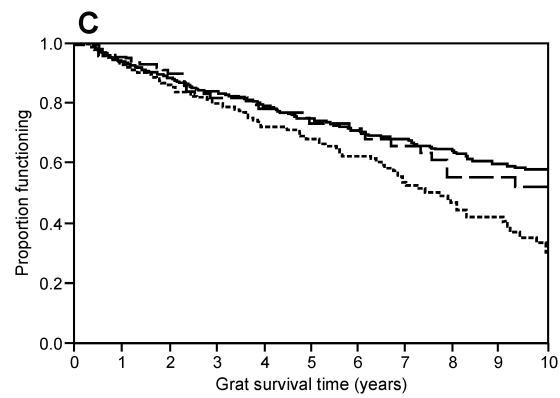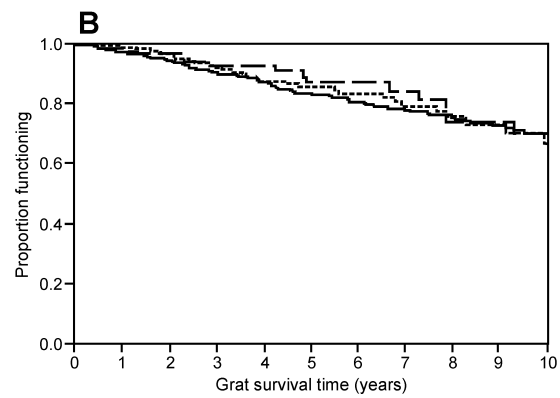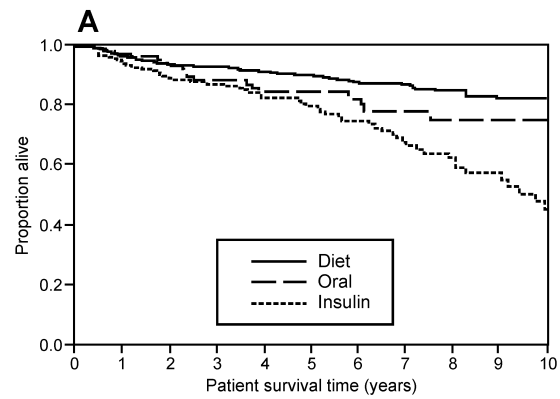Alternative Figures 1&2 (including actual graft survival) Kaplan Merier estimates according to level of HbA1c (Figure 1) and maximal non-fasting glucose level (Figure 2) stratified for quartiles of the respective parameter. Evaluated outcomes: patients survival (Figures A), functional graft survival (Figures B), actual graft survival (Figures C); p-values from log-rank test: Figure 1 A: 0.107, B: 0.143, C: 0.031; Figure 2 A: <0.001, B: 0.111, C: <0.001.

Fig. 1

Fig. 2

1st	140	137	122	95	45	31
2nd	126	93	89	56	31	16
3rd	138	102	70	48	25	15
4th	128	108	90	63	36	18

1st	179	300	248	188	111	67
2nd	181	88	56	34	21	12
3rd	181	93	79	51	27	5
4th	174	88	65	40	11	5
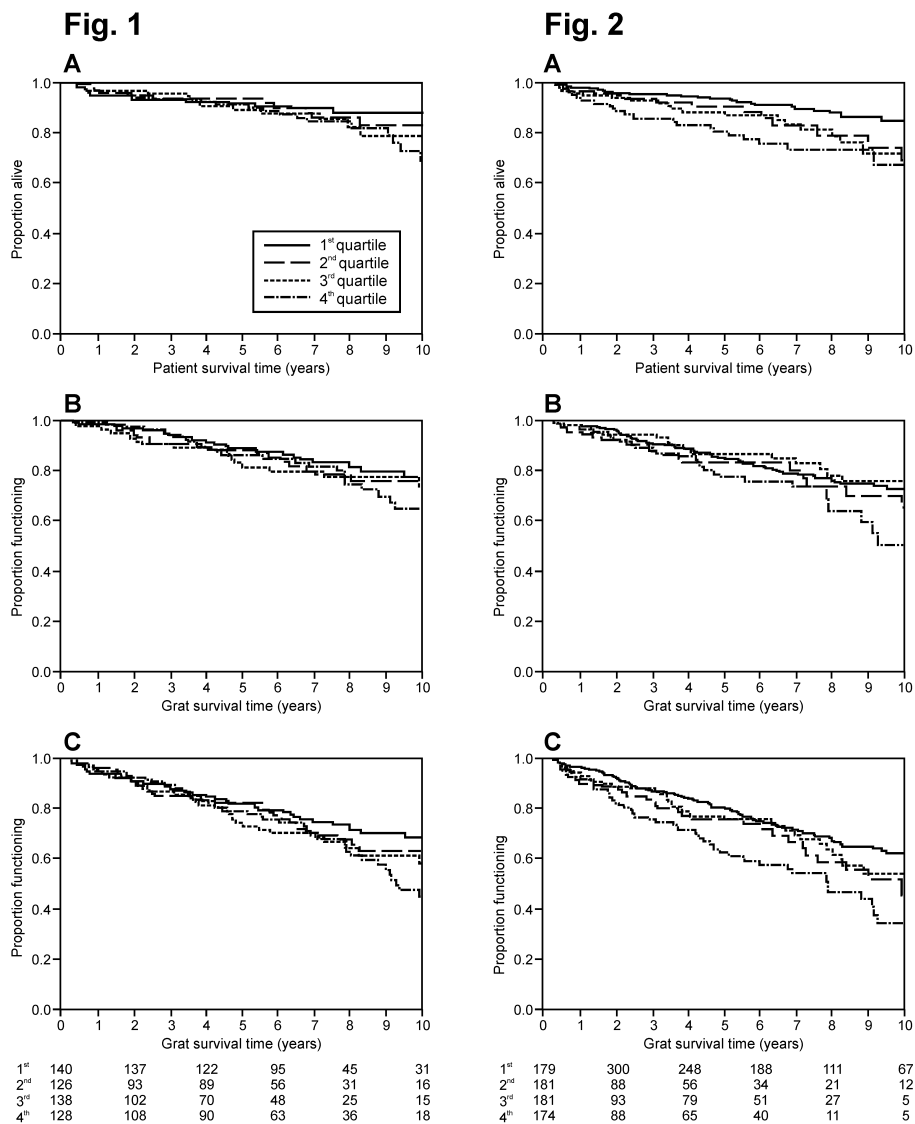
Alternative Figure 3 (including actual graft survival) Hazard ratios (HR) for quartiles of maximal non-fasting glucose levels (Figure A, adjusted for: number of antihypertensive drugs, total cholesterol, type of immunosuppressive therapy, year of transplantation, mean arterial pressure, and donor age); and HbA1c (Figure B, adjusted for: number of antihypertensive drugs, cerebro- and peripheral vascular disease, total cholesterol, type of immunosuppressive therapy, year of transplantation, cold ischemia time, mean arterial pressure, donor age) HR's >1 are associated with an increased likelihood of patient death or graft loss; * p-value for trend;
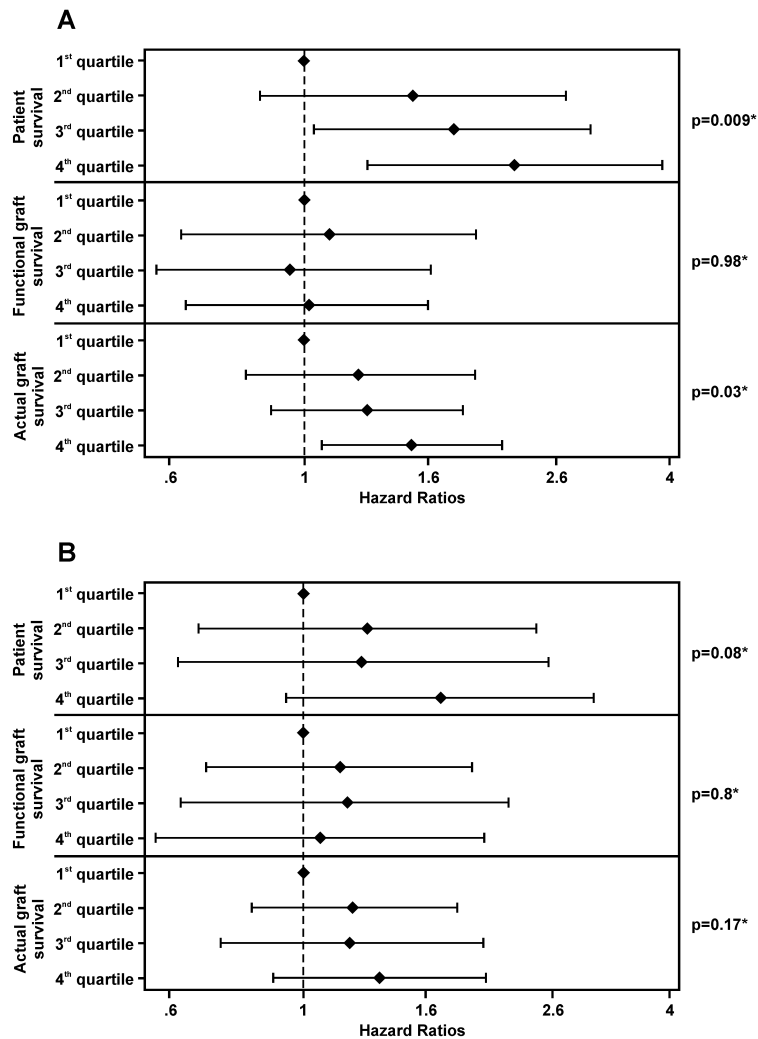
Alternative Figure 4 (including actual graft survival) Kaplan Merier estimates according to

treatment group. Evaluated outcomes: patients survival (Figures A), functional graft survival

(Figures B), actual graft survival (Figures C); p-values from log-rank test: A: <0.001, B: 0.825, C:

<0.001.

Diet	527	383	328	240	134	76
Oral	93	83	61	39	20	10
Insulin	178	151	101	67	37	17

Alternative Figure 5 (including actual graft survival) Hazard ratios (HR) derived from the analysis using marginal structural models (MSM) according to treatment group and outcome; HR's >1 are associated with an increased likelihood of patient death or graft loss;
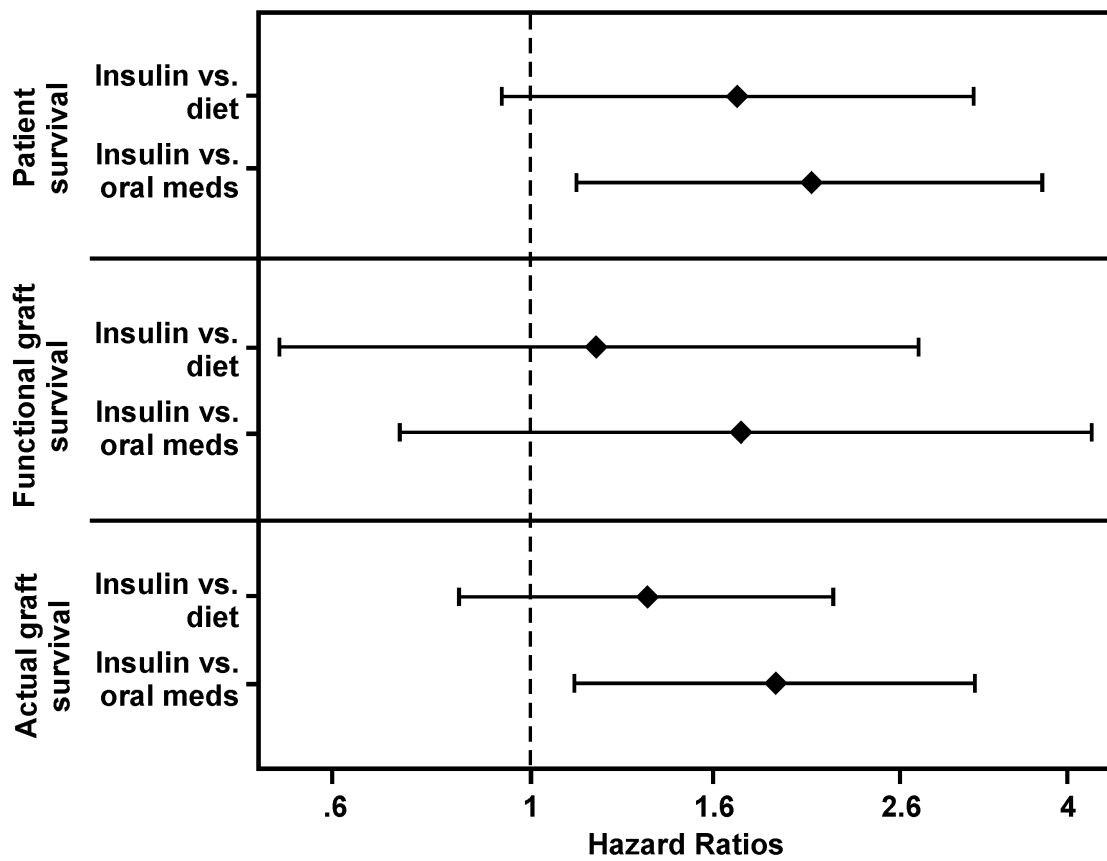
9. Cox analysis including the variables: "number of hospitalizations" and "primary indication for TX: diabetes"

The number of hospitalizations could influence the probability of detection of high plasma glucose levels and could also be related to higher risk of death or graft failure. Therefore, we conducted a sensitivity analysis including hospitalization in the multivariate model. Furthermore, this model also included the primary indication for transplantation (diabetes vs. other causes).

outcome	Parameter	Hazard Ratio	Lower 95% CL HR	Upper 95% CL HR	Pr > |t|
actual graft survival	glukose_max_q1	1.26	0.86	1.85	0.2408
actual graft survival	glukose_max_q2	1.24	0.86	1.77	0.2478
actual graft survival	glukose_max_q3	1.45	1.04	2.02	0.0280
actual graft survival	Trend	1.12	1.01	1.25	0.0334
functional graft surv	glukose_max_q1	1.09	0.67	1.78	0.7332
functional graft surv	glukose_max_q2	0.89	0.53	1.50	0.6570
functional graft surv	glukose_max_q3	1.01	0.63	1.60	0.9818
functional graft surv	Trend	0.99	0.85	1.15	0.8822
patient survival	glukose_max_q1	1.55	0.85	2.86	0.1529
patient survival	glukose_max_q2	1.76	1.07	2.90	0.0266
patient survival	glukose_max_q3	2.17	1.32	3.56	0.0022
patient survival	Trend	1.29	1.10	1.51	0.0021

We used the number of glucose measurements per year as a proxy of hospitalization. This number was distributed as follows: 25th percentile = 0, Median= 3, 75th percentile =15, 90th percentile = 36. Because of the skew distribution with a peak at 0, we have categorized into 0/1-15/>15, and assigned semiparametric values of 0/1/2 to these categories. When including this new variable as well as presence of diabetic nephropathy as primary indication of transplantation in the models for maximum glucose value per calendar year, we noticed higher risk for patients with more measurements in patient survival (HR=1.3, p=0.290), in functional graft survival (HR=2.9, p<0.001), and in actual graft survival (HR=2.0, p<0.001). The hazard ratios related to quartiles of maximum glucose were not altered by inclusion of number of measurements per year: comparing the highest to the lowest quartiles of maximum glucose, we estimated higher risk for patient death by 2.2fold (95% CI 1.3 to 3.6, p-value for trend 0.002, original result 2.2). The risk of actual graft loss (counting graft failure and death as events) increased by a factor of 1.4 when the same quartiles were compared (95% CI 1.02-2.02, p-value for trend 0.033, original result 1.5). Similar to our initial results, there was again no indication of correlation of maximum glucose with graft loss if additionally adjusted by number of measurements per year and diabetic nephropathy.