Glucose Metabolism Abnormalities After Renal Transplantation: Studies On Epidemiology, Mechanisms And Outcomes

Submission for M.D.

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DECLARATION

Except where indicated by specific reference, the work submitted in this thesis is the result of my own investigation and the views expressed are mine.

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No portion of the work presented has been submitted in substance for any other degree or award at this or any other university or place of learning, nor is being submitted concurrently in candidature for any degree or other award.

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ACKNOWLEDGEMENTS

Dedicated

To my parents

SUMMARY

Abnormal glucose metabolism, including new-onset diabetes after transplantation (NODAT), is a common complication following kidney transplantation. Better understanding of the causes, associations, prediction and outcomes of NODAT in the modern era of kidney transplantation is essential.

Our central hypothesis was that early NODAT is distinct from type 2 diabetes mellitus (T2DM), and is due to factors unique to the transplant setting, of which the predominant factor is the use of specific immunosuppressive agents (calcineurin inhibitors-CNIs), and that traditional risk factors for T2DM are the more significant factors late after transplantation.

In a series of observational studies, we found that recipient age, body mass index and baseline plasma glucose levels were associated with the development of NODAT, both early and late after transplantation. Exposure to tacrolimus and being transplanted in an older era were associated with early NODAT development, but had no effect on late NODAT. There was increasing insulin resistance but no compensatory increase in insulin secretion in patients developing NODAT, suggesting an effect of CNIs. In an observational study using paired oral glucose tolerance tests, there was worsening of glucose tolerance late after transplantation. Metabolic syndrome was a risk factor for this deterioration. Finally, in an epidemiological study, we show that immunosuppression regimens in Cardiff have evolved, with the introduction of induction therapy and tacrolimus as the CNI of choice. Blood tacrolimus levels, corticosteroid exposure and acute rejection rates were lower in a recent era of transplantation, as was the incidence of NODAT. NODAT developing within the first year, higher systolic blood pressure and higher serum creatinine level were all associated with increased mortality.

In conclusion, traditional T2DM risk factors are important in causing both early and late NODAT, with a strong influence from immunosuppressive agents early after transplantation. NODAT and other cardiovascular risk factors were associated with mortality. Therefore, less diabetogenic immunosuppressive regimes and interventions to reduce hyperglycaemia may not improve mortality unless other cardiovascular factors are also managed simultaneously.

Contents

PUBLICATIONS RESULTING FROM THIS WORK

1. Nagaraja P, Sharif A, Ravindran V, Baboolal K. Long-term progression of abnormal glucose tolerance and its relationship with the metabolic syndrome after kidney transplantation. Transplantation. 2014 Mar 15;97(5):576-81.

2. Nagaraja P, Ravindran V, Morris-Stiff G, Baboolal K. Role of insulin resistance indices in predicting new-onset diabetes after kidney transplantation. Transpl Int. 2013 Mar;26(3):273-80.

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LIST OF PRESENTATIONS TO LEARNED SOCIETIES

1. Pathophysiology, epidemiology and outcomes of NODAT – Oral presentation at the Welsh Association of Renal Physicians and Surgeons, June 2014

2. New-onset diabetes after transplantation: pathophysiology and long-term impact in renal allograft recipients – Oral presentation at the American Transplant Congress, Philadelphia, May 2011

3. Early development of NODAT is associated with impaired long-term survival in renal allograft recipients – Oral presentation at the British Transplantation Society Meeting, Bournemouth, March 2011

4. Declining incidence of NODAT after kidney transplantation with newer immunosuppression protocols - Poster of distinction at the American Transplant Congress, Seattle, May 2013

5. A longitudinal study of the progression of abnormal glucose tolerance in renal allograft recipients - Poster of distinction at the American Transplant Congress, Boston, June 2012

6. Withdrawal of calcineurin inhibitors for chronic allograft nephropathy: Effect on allograft function and plasma glucose – Poster presentation at the British Transplantation Society Meeting, Glasgow, February 2012

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LIST OF ABBREVIATIONS

ADA	American Diabetes Association
APKD	Adult Polycystic Kidney Disease
AR	Acute Rejection
ATG	Anti-Thymocyte Globulin
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
CMV	Cytomegalovirus
CNI	Calcineurin Inhibitor
CTU	Cardiff Transplant Unit
CV	Cardiovascular
CVA	Cerebrovascular Accident
DBD	Donation after Brainstem Death
DCD	Donation after Circulatory Death
DCGL	Death Censored Graft Loss
DCGS	Death Censored Graft Survival
DM	Diabetes Mellitus
DPP-4	DiPeptidyl Peptidase-4
eGFR	Estimated Glomerular Filtration Rate
EGP	Endogenous Glucose Production
ESRF	End Stage Renal Failure
FPG	Fasting Plasma Glucose
FPI	Fasting Plasma Insulin
GLP-1	Glucagon Like Peptide-1

HDL	High Density Lipoprotein
HOMA	Homeostasis Model Assessment
HOMAsec	HOMA secretion index
HR	Hazard Ratio
IDF	International Diabetes Federation
IFG	Impaired Fasting Glucose
IFTA	Interstitial Fibrosis / Tubular Atrophy
IGT	Impaired Glucose Tolerance
IL	Interleukin
IR	Insulin Resistance
ITT	Intention To Treat
IVGTT	Intravenous Glucose Tolerance Test
K-M	Kaplan-Meier
KTR	Kidney Transplant Recipient
LDL	Low Density Lipoprotein
MMF	Mycophenolate Mofetil
MS	Metabolic Syndrome
NEFA	Non-Esterified Fatty Acids
NFAT	Nuclear Factor of Activated T-cells
NGT	Normal Glucose Tolerance
NHSBT	National Health Service Blood and Transplant
NODAT	New Onset Diabetes After Transplantation
OGTT	Oral Glucose Tolerance Test
OR	Odds Ratio
QUICKI	Quantitative Insulin Sensitivity Check Index

S.	Serum
SD	Standard Deviation
TG	Transplant Glomerulopathy
UHW	University Hospital of Wales
UK	United Kingdom
WHO	World Health Organisation
WHR	Waist Hip Ratio

CHAPTER 1: INTRODUCTION

1.1   SCOPE OF KIDNEY TRANSPLANTATION IN THE UK

Kidney transplantation is recognised as the treatment of choice for patients with end-stage renal failure (ESRF) as it not only improves survival, but also improves quality of life (1, 2). The first successful living donor kidney transplantation in the United Kingdom (UK) was carried out in 1960 and the first transplant of a deceased donor organ was performed in 1965. Since then, there has been a steady increase in the number of kidney transplants being performed in the UK. The National Health Service Blood and Transplant (NHSBT) Annual Report from 2014 reported that in the financial year 2013-2014, there were 1880 deceased donor and 1050 living donor kidney-only transplant procedures performed in the UK, indicating an increase from 1313 deceased donor and 386 living donor procedures in 2001-2002 (3). Tremendous developments have taken place in transplant immunology and anti-rejection drug discovery, leading to excellent allograft and patient survival rates. Figure 1.1a and 1.1b show the Kaplan-Meier estimates of death-censored graft survival (DCGS) after first adult kidney-only transplant from donors after brain death (DBD) and living donors respectively in the UK from 1997 to 2009 (4). Clearly, there have been significant improvements in graft survival over time. According to the latest figures, the probability of a kidney allograft from any deceased donor surviving in the UK at one year is 93% (95% CI 93-94) and at five years is 86% (95% CI 85-87). Outcomes from living donor kidney transplantation are even better; the probability of one-year graft survival is 96% (CI 95-97) and of five years is 89% (95% CI 88-90) (3).

Figure 1.1 – (a) Long-term graft survival in adult (>18 years) recipients of first kidney-only transplant from donors after brain death (b) Long-term graft survival in adult (>18 years) recipients of first kidney only transplant from living donors

Long-term graft survival after first adult kidney only transplant from donors after brain death, 1 January 1997 – 31 December 2009
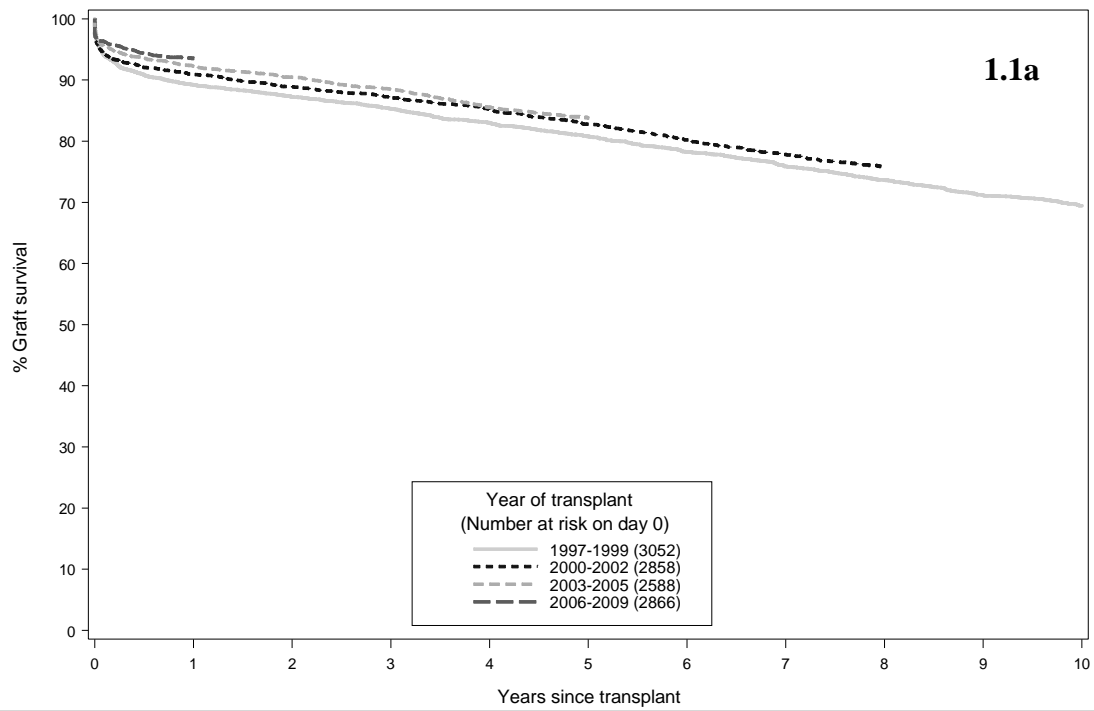
Source: Transplant activity in the UK, 2010-2011, NHS Blood and Transplant

Long-term graft survival after first adult living donor kidney only transplant in the UK, 1 January 1997 – 31 December 2009

1.1b

% Graft survival

Year of transplant
(Number at risk on day 0)
1997-1999 (525)
2000-2002 (830)
2003-2005 (1141)
2006-2009 (2607)

Years since transplant

Source: Transplant activity in the UK, 2010-2011, NHS Blood and Transplant

3
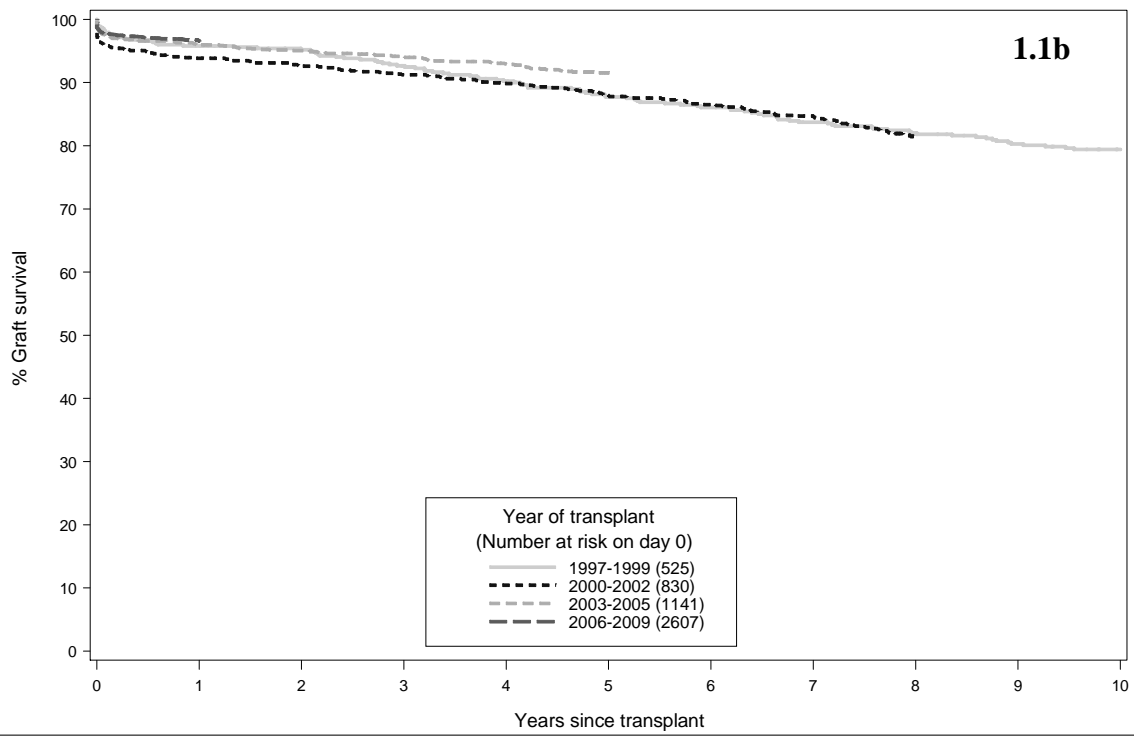
1.2   SCOPE OF KIDNEY TRANSPLANTATION IN CARDIFF

The kidney transplantation program started in Cardiff in the early 1970s and has grown to a great extent in terms of size and scope. At the Cardiff Transplant Unit (CTU) of University Hospital of Wales (UHW), there is now a well-established living donor and deceased donor kidney transplant program. In recent years, there has been an increase in donation after circulatory death (DCD) donor transplants. There is also a desensitisation program for ABO-incompatible and HLA-incompatible living donor pairs to enable transplantation in potential recipients who would otherwise be difficult to match in the national deceased donor program. A total of 150 kidney transplants were performed in the CTU in 2011 and the outcomes have been excellent. For recipients transplanted in the CTU, the risk-adjusted five year graft and patient survival estimates of 87% and 89% for deceased donor transplants and 89% and 98% for living donor transplants compare favourably to the corresponding UK national figures (deceased donor 86% and 89%, and living donor 91% and 95% respectively) (3). Risk adjustment was for factors known to affect graft and patient survival (graft year, donor age, donor type, donor cause of death, recipient age, waiting time to transplant, primary renal disease, HLA mismatch group and recipient ethnicity).

1.3   CHALLENGES IN THE LONG-TERM MANAGEMENT OF KIDNEY TRANSPLANT RECIPIENTS

Despite a significant reduction in the incidence of acute rejection (AR) in recent years and an improvement in short-term graft survival, long-term graft survival has not improved significantly (5). Authors of a large US study who made this observation hypothesised that this discordance might be related in part to a higher proportion of AR episodes which have not resolved with full functional recovery in the current era (5).

Interstitial fibrosis and tubular atrophy (IFTA, previously termed chronic allograft nephropathy) and the increasingly well-recognised condition of transplant glomerulopathy (TG) have emerged as the main causes of graft failure in recent years (6-11). IFTA can be caused by a variety of non-immunological causes including hypertension, calcineurin inhibitor (CNI) toxicity, chronic urinary obstruction and viral infections (11). Various immunosuppression regimens have been tried in managing IFTA with varying degrees of success (12). Chronic antibody-mediated rejection is a major cause of TG, which has poor graft prognosis (8, 9). Currently, no consensus exists regarding its optimal treatment.

Death with a functioning kidney is also a major reason for graft loss, with cardiovascular disease (CVD) being the leading cause of death (13-15), especially in patients with the metabolic syndrome (MS) (16). Other significant challenges include infections, malignancies and new-onset diabetes after transplantation (NODAT) (17, 18).

Before examining the pathophysiology of NODAT, we will first review below the current understanding on normal glucose metabolism and the pathophysiology of type 2 diabetes mellitus (T2DM).

1.4 NORMAL GLUCOSE METABOLISM AND INSULIN SECRETION

Figure 1.2 summarises the processes involved in normal glucose homeostasis (19). In the fasting state, endogenous glucose production (EGP) is mainly through hepatic glycogenolysis and gluconeogenesis. Glucose uptake in the fasting state is mainly by insulin-independent and glucose-dependent tissues such as the brain. A low plasma insulin-to-glucagon ratio aids the above-mentioned processes and prevents hypoglycaemia during fasting. Tissues such as the heart and skeletal muscle are mainly provided with non-glucose nutrients such as non-esterified fatty acids from adipose tissue lipolysis. After a carbohydrate meal, glucose

concentration in the blood rises, which stimulates insulin secretion by pancreatic islet β-cells and suppresses glucagon secretion from α-cells. EGP is suppressed and glucose uptake into insulin-sensitive peripheral tissues such as the heart, skeletal muscle, and adipose tissue is triggered, thus increasing the rate of glucose disposal from blood. The action of glucose on islet cells to increase insulin secretion and suppress glucagon secretion is facilitated by gut peptides called incretins [eg. glucagon-like peptide-1 (GLP-1]. Insulin then suppresses adipose tissue lipolysis and anabolic metabolism is promoted. The ultimate result is that blood glucose concentration falls towards the fasting level within two hours.

Normoglycaemia is maintained by the balanced interplay between insulin action and insulin secretion. The normal pancreatic β-cell can adapt to changes in insulin action: a decrease in insulin action (i.e. reduced sensitivity) is accompanied by upregulation of insulin secretion and vice versa. A hyperbolic relationship has been described between normal β-cell function and insulin sensitivity (Figure 1.3) (20, 21).

Insulin is synthesized as preproinsulin in the ribosomes of the rough endoplasmic reticulum of islet β-cells, which is then cleaved to proinsulin. Proinsulin is transferred to the Golgi apparatus where it is packaged into secretory granules close to the cell membrane. Proinsulin is cleaved into equimolar amounts of insulin and C-peptide in the secretory granules. At the time of secretion, these secretory granules fuse with the cell membrane and this leads to the exocytosis of insulin, C-peptide, and proinsulin.

Figure 1.2 – An overview of normal glucose homeostasis [Adapted from Nolan et al. (19)] (EGP – endogenous glucose production, GLP-1 – glucagon-like petide-1)
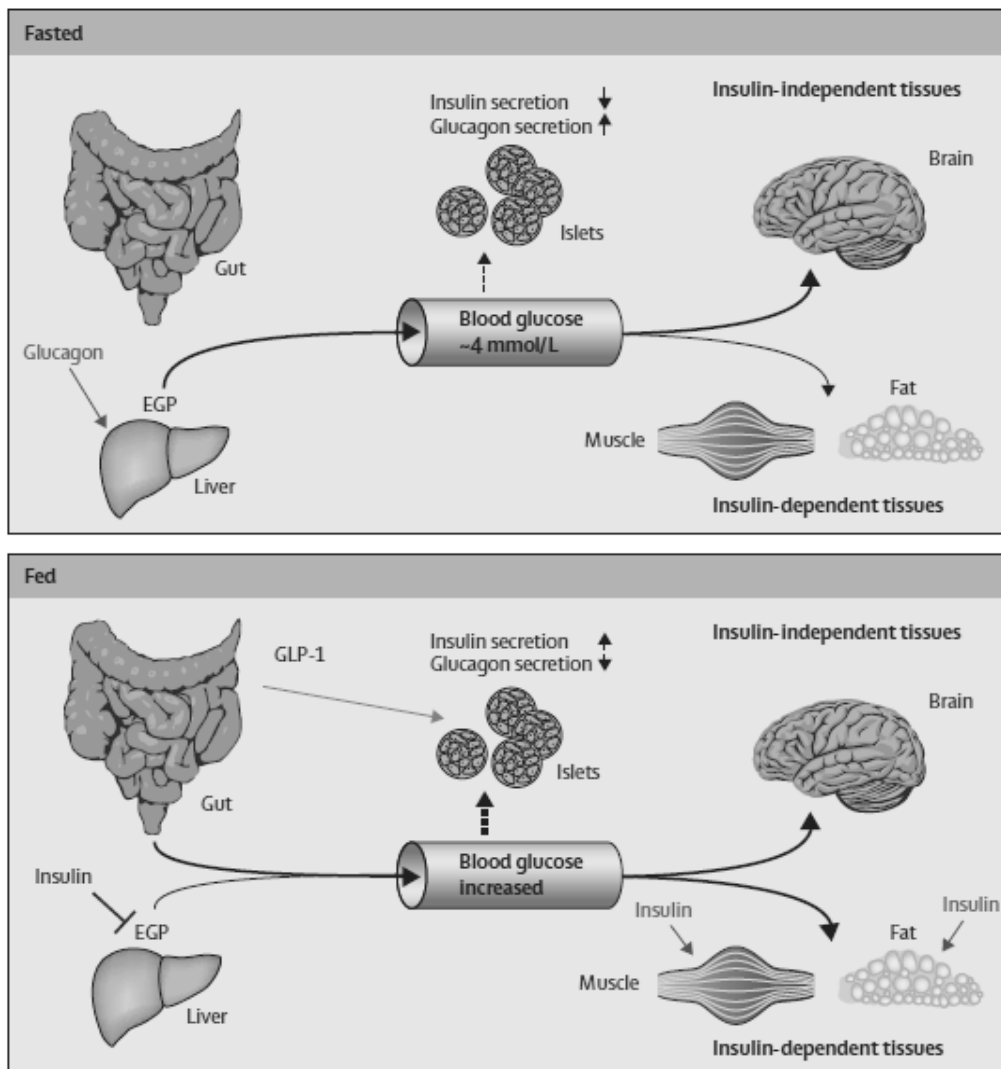
Figure 1.3 - Hyperbolic relation between β-cell function and insulin sensitivity (Adapted from Stumvoll et al. (22), T2DM – type 2 diabetes mellitus, IGT – impaired glucose tolerance, NGT – normal glucose tolerance)
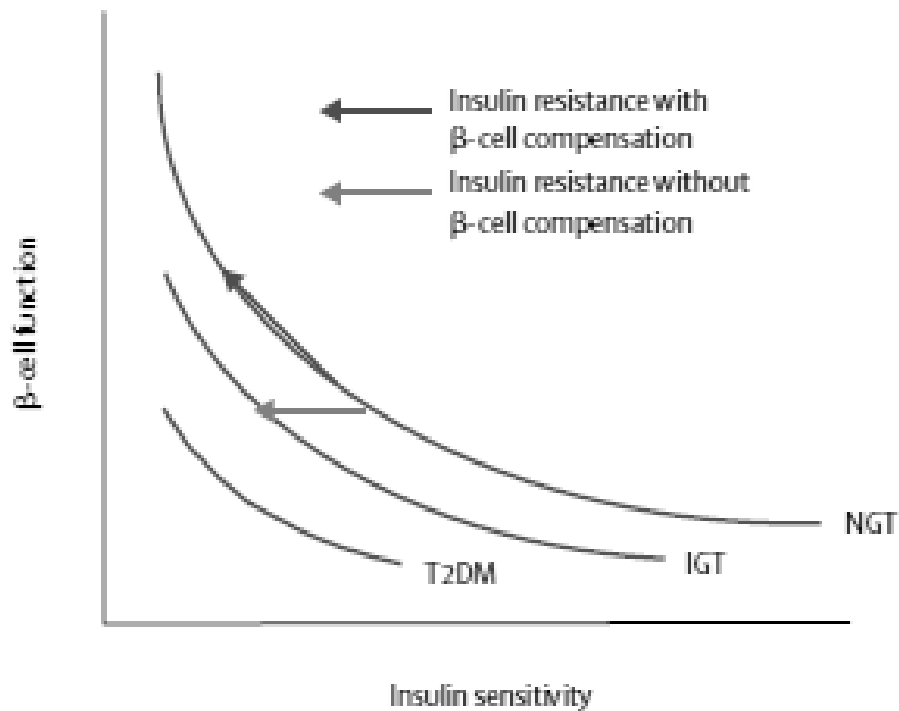
1.4.1 ROLE OF CALCINEURIN/NFAT PATHWAY IN THE REGULATION OF B-CELL FUNCTION

CNIs (tacrolimus and ciclosporin) are the commonest immunosuppressive agents used in renal transplantation. They act by binding to their respective cytoplasmic receptors (cyclophilin and FKBP), and these bound complexes in turn inhibit the phosphatase activity of calcineurin (23). This results in a blockage to the translocation of nuclear factor of activated T-cells (NFAT) from the cytoplasm to the nucleus. The end result is an inhibition of T-cell activation and cytokine gene transcription.

In addition to its important role in T-lymphocyte activation, calcineurin is also known to influence transcriptional regulation in a variety of non-immune cells, including in tissues involved in glucose metabolism (24, 25). Therefore, its inhibition by CNIs could potentially have wide-ranging effects. The mechanisms of these effects on pancreatic β-cells have been widely studied, as described below (26).

In human cell culture studies using HIT-T15 cell lines (a glucose-responsive, insulin-secreting islet cell line), tacrolimus did not have an acute effect on insulin secretion, but caused a reversible time- and dose-dependent decrease in insulin secretion (27). In these studies by Redmon et al., in the presence of tacrolimus, there was a decrease in insulin secretion as well as a dose-dependent decrease in cell insulin content, insulin mRNA levels, and expression of a human insulin promoter gene. Lawrence et al. showed that calcineurin regulates insulin gene transcription via a mechanism involving NFAT interaction with specific elements within the insulin promoter (28). Using studies in mice, they also showed that disruption of this pathway in vivo under chronic tacrolimus treatment causes diabetes. Based on results from their studies, they proposed that there are at least two distinct cell signalling pathways that affect insulin gene transcription: a rapamycin-sensitive pathway and a calcium-dependent (tacrolimus-sensitive) pathway (Figure 1.4). The rapamycin-sensitive pathway requires the glucose-induced calcium-dependent pathway to exert its full effect. Finally, Heit et al., using in vivo studies in mice established that calcineurin/NFAT signalling regulates multiple factors that control β-cell growth and function (29). They showed that mice with a β-cell-specific deletion of a calcineurin phosphatase regulatory subunit develop age-dependent diabetes characterized by decreased β-cell proliferation and mass, reduced pancreatic insulin content and hypoinsulinaemia. Such β-cells also had a reduced expression of regulators of β-cell proliferation.

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In summary, these studies strongly suggest that the principal mechanism of NODAT caused by CNIs could be the disruption of the calcineurin/NFAT pathway in β-cells leading to impaired insulin synthesis and a reduction in β-cell mass.

Figure 1.4 – A scheme proposed by Lawrence et al. for the activation of the insulin gene promoter by glucose [adapted from Lawrence et al (28)] (Glc – glucose, Cn – calcineurin, NFAT – nuclear factor of activated T cells, FK506 – tacrolimus, RAP – sirolimus)
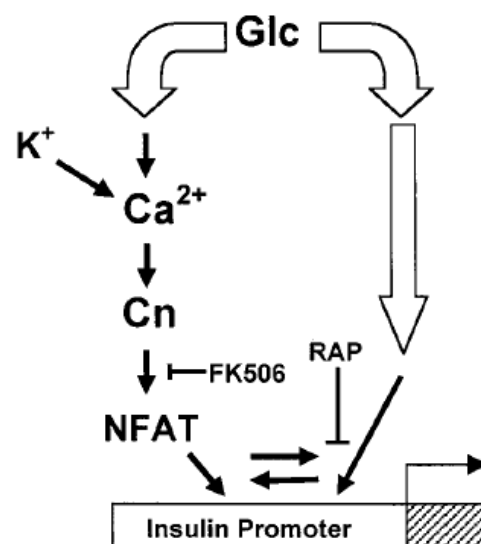
1.5 PATHOPHYSIOLOGY OF TYPE 2 DIABETES MELLITUS

Type 2 diabetes mellitus (T2DM) is characterized by hyperglycaemia, insulin resistance, and a relative impairment in insulin secretion. Its prevalence has risen significantly in the recent decades, largely due to rising obesity and sedentary lifestyles (30, 31). Patients with T2DM

usually have a combination of varying degrees of insulin resistance and relative insulin deficiency (32). As we have seen in the previous section, there is a hyperbolic relationship between insulin sensitivity and insulin secretion. Deviation from this hyperbola in patients with impaired glucose tolerance and T2DM occurs when β-cell function is inadequately low for a specific degree of insulin sensitivity (Figure 1.3). The importance of β-cell dysfunction in the pathogenesis of T2DM has been verified in several large longitudinal studies, three of which are summarised below (33-35).

In a series of studies in Pima Indians in the USA, Weyer et al. measured insulin action, insulin secretion, and endogenous glucose production (EGP). Seventeen subjects experienced a deterioration in glucose tolerance from normal (NGT) to impaired (IGT) to diabetes over 5.1 ± 1.4 years. Transition from NGT to IGT was associated with an increase in body weight, a decline in insulin-stimulated glucose disposal, and a decline in the acute insulin secretory response (AIR) to intravenous glucose, but no change in EGP. Progression from IGT to diabetes was accompanied by a further increase in body weight, further decreases in insulin-stimulated glucose disposal and AIR, and an increase in basal EGP. In contrast, 31 subjects who retained NGT over a similar period also gained weight, but their AIR increased with decreasing insulin-stimulated glucose disposal.

In a cohort study of British civil servants (Whitehall II study), over a median period of 8.2 years, 505/6538 subjects were diagnosed with T2DM (34). There was a steep decline in insulin sensitivity (or an increase in insulin resistance) starting from five years prior to the diagnosis of DM (Figure 1.5). β-cell function increased between four and three years prior to diagnosis, and then fell until diagnosis to a level below that of non-diabetic controls.

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Figure 1.5 – Trends of insulin sensitivity and secretion in subjects developing T2DM and controls in the Whitehall II study [Adapted from Tabák et al. (34)]
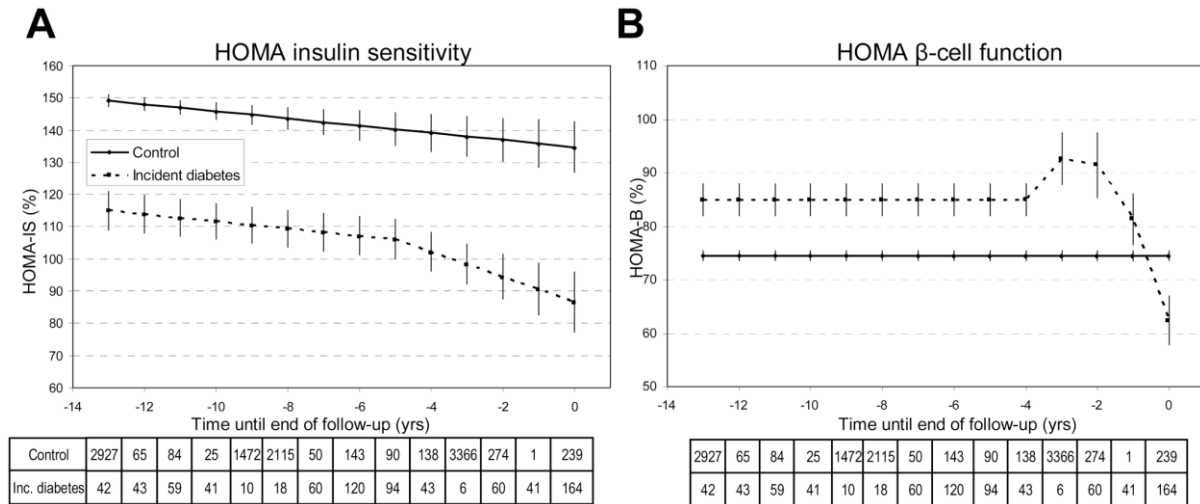
In a study amongst non-diabetic Mexican Americans, Haffner et al. noted that subjects with markers of a higher baseline insulin resistance and lower insulin secretion had a higher incidence of T2DM over a 7-year period (35). This was the case both in subjects with NGT and IGT.

Taken together, these studies suggest a decompensation in β-cell function in the context of decreasing insulin sensitivity in subjects developing T2DM. The current understanding of the mechanisms involved in reducing insulin sensitivity and insulin secretion are reviewed below.

1.5.1 DECREASING INSULIN SENSITIVITY OR INCREASING INSULIN RESISTANCE

Decreasing insulin sensitivity is said to occur when the biological effects of insulin are less than expected for both glucose disposal in skeletal muscle and suppression of EGP in the

liver (36). There is lack of suppression of EGP in subjects with T2DM, most likely due to hepatic insulin resistance (33). The mechanism of this insulin resistance is thought to involve adipocytes, which are known to secrete various hormones (adipokines), inflammatory cytokines and non-esterified fatty acids (NEFAs) (37). These chemicals in turn modulate insulin action. Free fatty acids have been shown to increase insulin resistance in healthy volunteers by reducing peripheral glucose uptake and increasing hepatic glucose output (38). Increasing blood levels of NEFAs therefore aggravate insulin resistance in peripheral tissues as well as in the liver (39).

In contrast, adiponectin is an adipokine which is known to have an insulin-sensitising effect in liver and skeletal muscle (40, 41). Its levels are reduced in visceral obesity and states of insulin resistance. It has been shown to act via AMP-kinase signalling, which is implicated in increased glucose utilization, fatty acid oxidation and inhibition of lipolysis (42). Another adipokine with wide-ranging effects on energy homeostasis and glucose metabolism is leptin (43). Studies on isolated rat adipocytes have suggested that leptin may alter the effect of insulin on these cells (44, 45). Also, there is evidence from in vitro animal cell studies demonstrating a reduction in insulin release from pancreatic β-cells under the influence of leptin (46-48). Other adipokines such as the fibroblast growth factor 21 and dipeptidyl peptidase-4 are also known to have effects on glucose metabolism (49, 50).

Several inflammatory cytokines, including IL-6, derived from the adipose tissue have been correlated with obesity and insulin resistance (37). Interestingly, peripheral blood mononuclear cell-derived IL-6 levels have been found to be elevated in renal transplant recipients during episodes of acute rejection (51, 52). However, there are no studies linking episodes of rejection with insulin resistance. Also, only treated rejection episodes have been

associated with an increased incidence of NODAT (53, 54). Therefore, it is not clear if there is a direct relationship between acute rejection and glucose metabolism, or if this relationship is due to enhanced immunosuppression, especially with steroid boluses.

1.5.2 β-CELL DYSFUNCTION

Impaired insulin secretion is present in people destined to develop T2DM well before the onset of overt hyperglycaemia. This has been shown in people with impaired fasting glucose (55) and in healthy offspring of patients with T2DM (56). Moreover, as explained in section 1.6, there is worsening β-cell function in subjects with worsening glucose tolerance (33-35). This failure in the compensation of β-cells to produce an adequate quantity of insulin in the face of increasing insulin resistance is thought to precipitate overt hyperglycaemia. The mechanisms involved in this decompensation are reviewed below.

The first step is the production of "susceptible β-cells" which decompensate in the presence of continuing nutrient overload and insulin resistance (57). This susceptibility is related to various genetic and environmental factors. Several genome-wide association studies have identified diabetes-susceptible loci in genes involved in β-cell growth and development and insulin synthesis (58-60). Table 1.1 shows examples of some genes which are associated with reduced insulin secretion in humans (61). The mechanisms by which polymorphisms in these candidate genes cause β-cell dysfunction is not entirely clear. For example, one gene that has been associated with the development of T2DM is *TCF7L2*, which is implicated in cell proliferation and the incretin effect. In the risk-bearing T-allele carriers of a polymorphism of *TCF7L2,* there is impaired insulin secretion and incretin effects, and enhanced rate of hepatic glucose production (62). In this prospective study by Lyssenko et al., *TCF7L2* expression in human islets was increased 5-fold in patients with T2DM

compared to non-diabetic individuals, particularly in carriers of the risk TT genotype. Overexpression of *TCF7L2* in human islets was associated with reduced glucose-stimulated insulin secretion. On the other hand, in a study by Shu et al., TCF7L2 protein expression was found to be markedly reduced in human type 2 diabetic islets (63). Therefore, the correlation between TCF7L2 genotype and protein expression and the effects on human islet cells still need to be clarified.

Examples of environmental factors posing a risk for T2DM include intra-uterine growth retardation and maternal hyperglycaemia during pregnancy (64-67). Epigenetic mechanisms have been proposed to explain the interaction between the intra-uterine environment and the risk of future non-communicable diseases such as T2DM in the offspring (68).

The next step in the decompensation of insulin secretion is the initiation of β-cell dysfunction. Glucotoxicity, lipotoxicity and glucolipotoxicty have all been shown to be detrimental to β-cell survival. Glucotoxicity is a concept proposed to explain the adverse effect of hyperglycaemia on the cellular function of β-cells, which in turn causes impaired insulin secretion over time (69, 70). The production of reactive oxygen species due to oxidative glucose metabolism is thought to damage cellular components leading to impaired insulin gene transcription and also β-cell apoptosis. More recently, lipotoxicity has also been proposed as a mechanism of β-cell dysfunction (71). We have seen earlier that blood concentration of NEFAs is elevated in obesity due to inhibition of lipolysis. In the presence of glucose, the oxidation of these fatty acids is decreased in β-cells leading to the accumulation of long-chain acyl coenzyme A, which on down-stream signalling can lead to cell damage (72).

Therefore, progressive insulin deficiency due to β-cell failure is perpetuated by glucose- and NEFA-induced toxicity. The role of islet amyloid polypeptide in initiating and continuing β-cell dysfunction is still not clear.

Table 1.1 – Genes/loci associated with reduced insulin secretion in humans (adapted from Marchetti et al. (61)

Author (Reference)	Genes
Nielson et al. (73)	KCNJ11
Saxena et al. (74)	TCF7L2
Staiger et al. (75)	SLC30A8
Steinthorsdottir et al. (76)	CDKAL1
Grarup et al. (77)	JAZF1, CDC123/CAMK1D, TSPAN 8
Sparso et al. (78)	WSF1
Staiger et al. (79)	MTNR1B
Holmkvist et al. (80)	KCNQ1
Saxena at al. (81)	GCKR, ADCY5, TCF7L2, VPS13C, GIPR

1.6 PATHOPHYSIOLOGY OF POST-TRANSPLANT HYPERGLYCAEMIA AND NODAT

Similar to T2DM, both insulin resistance and insulin hyposecretion are present in kidney transplant recipients (KTRs) with NODAT (82, 83). This was first demonstrated by Ekstrand et al. who used gold standard clamp methods for measuring insulin sensitivity and insulin secretion (82). They found significantly more insulin resistance and reduced first and second phase insulin secretion in patients with NODAT compared with healthy controls. Several subsequent studies have attempted to further delineate the relative importance of insulin resistance and insulin deficiency in KTRs.

Insulin resistance is present in KTRs who develop post-transplant hyperglycaemia and NODAT (82, 84). In a comparative study, Midtvedt et al. performed hyperinsulinaemic clamp studies to estimate insulin sensitivity and used the 1-hour post-glucose load serum insulin level as a measure of insulin response (84). All 46 KTRs in this study were on ciclosporin and were examined 12-14 weeks after transplantation. They found that patients with IGT and NODAT had a significantly lower insulin sensitivity compared to patients with NGT. Furthermore, insulin response was similar in NGT and IGT patients whereas NODAT patients had a lower insulin response compared to both NGT and IGT patients.

Hjelmesaeth et al. performed a prospective study in which 167 KTRs underwent an OGTT 10 weeks (baseline) and 12 months after transplantation (83). Indices of insulin sensitivity and secretion were derived from glucose and insulin values obtained during the OGTT. They found a significant stepwise impairment of both insulin sensitivity and insulin secretion, in parallel with decreasing glucose tolerance from NGT to NODAT. Moreover, those patients who improved from IGT/NODAT to NGT during the course of the study had higher insulin secretion at baseline than those who did not improve.

Nam et al. utilised OGTT-derived area-under-curve insulin (AUCinsulin) as a measure of insulin secretory capacity, and the insulin sensitivity index (ISI) derived from a short insulin tolerance test (ITT) as a measure of insulin sensitivity (85). They performed OGTT and ITT on 114 patients, one week pre-transplant (all living donor) and 9-12 months post-transplant to determine the pathophysiology of NODAT. All patients had normal glucose tolerance on the pre-transplant OGTT. This was a significant strength of the study, which accurately excluded patients with IGT and T2DM. Their significant findings were as follows:

1. Despite being within the normal range, pre-transplant fasting plasma glucose levels were incrementally higher in those patients who went on to develop IGT and NODAT post-transplantation, than those who remained in NGT (mean 4.3 mmol/l, 4.8 mmol/l and 5.3 mmol/l, $p < 0.02$).

2. Those patients who eventually developed NODAT by 9-12 months had a significantly lower pre-transplant insulin secretion than those who remained non-diabetic, despite having similar insulin sensitivity pre-transplantation.

3. Insulin sensitivity increased after transplantation in all groups but insulin secretion remained lower in the NODAT group compared to the non-diabetic groups.

3. In the subgroup of patients not gaining weight after transplantation, insulin sensitivity was similar amongst non-diabetics and those developing NODAT at 12 months, whereas weight gaining NODAT patients had a lower insulin sensitivity compared to weight gaining non-diabetics (Figures 1.6a and 1.6b).

Figure 1.6– a. Insulin response post-transplantation derived using OGTT and b. Insulin sensitivity post-transplantation derived using the short insulin tolerance test (From Nam et al. (85))(# denotes p<0.05 compared with the NGT group)
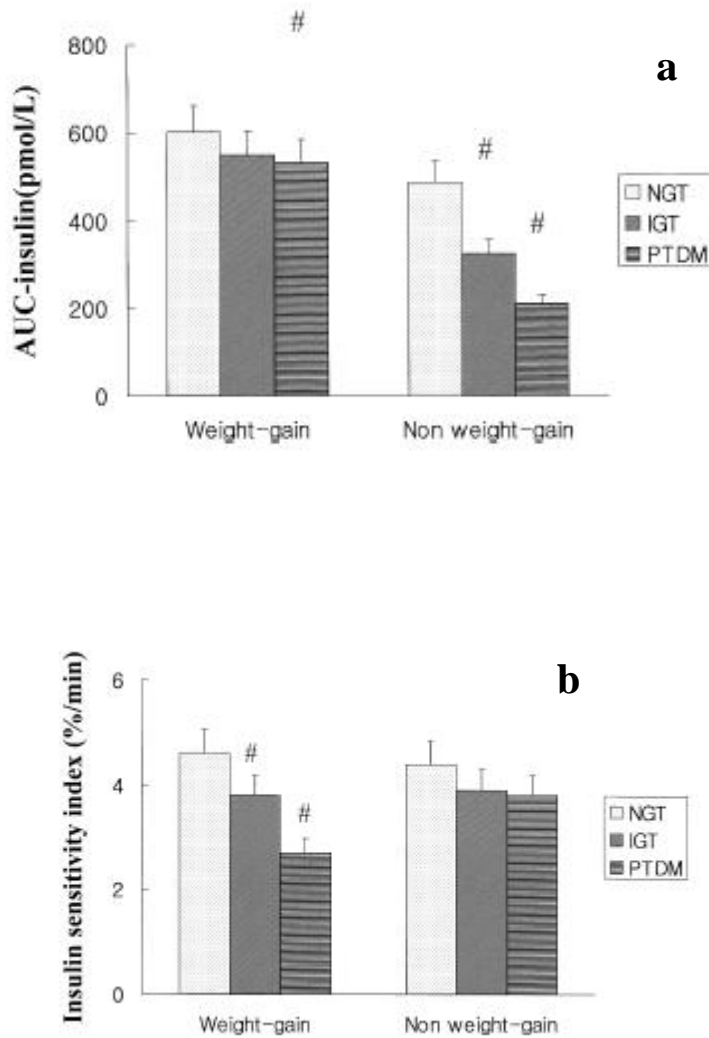
In conclusion, the above studies demonstrate that although insulin resistance is present in KTRs developing NODAT, a defect in insulin secretion seems to play the dominant role in its causation. However, all the above studies examined the pathophysiology of NODAT and glucose intolerance early after transplantation ($\leq$ 12 months). This is the period when patients are exposed to a higher level of immunosuppression in the form of CNIs or steroids. Therefore, it is plausible that the stress on β-cells is highest during this period. This is due to

a direct effect of CNIs on β-cells and indirectly through increased insulin resistance from the use of steroids which demands a higher insulin response.

In the period late after transplantation, when the risk of acute rejection is lower, and the levels of immunosuppression are reduced, there are no studies examining glucose intolerance and its progression. Studying glucose metabolism in this period is important, as we shall see later that there is a cumulative incidence of NODAT even late after transplantation.

1.7 MEASURES OF INSULIN SENSITIVITY AND SECRETION

Various methods of measuring insulin sensitivity and secretion that have been developed over time are reviewed below. They vary in their complexity, accuracy and cost. The "clamp" studies are considered gold-standard methods which can estimate stimulated insulin resistance and insulin secretion (86). Other techniques such as HOMA estimate the basal insulin resistance.

1.7.1 EUGLYCAEMIC CLAMP

First described by DeFronzo et al. in 1979, in this method, plasma glucose concentration is maintained constant (e.g. 5 mmol/l) by closely titrating glucose infusion against a fixed-rate insulin infusion (86). Once steady state is reached, the degree of insulin sensitivity is directly related to the amount of glucose necessary to maintain the required plasma glucose concentration (87).

1.7.2 HYPERGLYCAEMIC CLAMP

In this technique, plasma glucose concentration is maintained high at a pre-determined level (e.g.13 mmol/l) through a glucose infusion. The first-phase insulin response is estimated from the immediate plasma insulin concentration rise after glucose infusion (usually the first

10-minute interval). The second-phase insulin response is estimated from the more gradual and later plasma insulin concentration rise after glucose infusion (10-120 minute interval) (86). 1.7.3 Minimal model analysis and the frequently sampled intravenous glucose tolerance test (FSIVGTT)

Bergman et al. developed a minimal-model analysis based on the concept that there are two mechanisms for glucose disposal from plasma: 1 – insulin sensitivity and 2 – glucose effectiveness, which is the effect of glucose to increase the uptake of glucose and suppress endogenous output independent of the action of insulin (20). Individual patient data on plasma glucose and insulin derived from an FSIVGTT are used in a computer program to compute estimates of insulin sensitivity and secretion (88). In the FSIVGTT, subjects are given an IV bolus of glucose, followed by frequent sampling of blood over 240 minutes to measure plasma glucose, C-peptide, and insulin concentrations. The glucose load triggers a variable, biphasic insulin secretory response which can be estimated using the computer program.

Clamp methods and the FSIVTT are labour-intensive, complex and not suitable for large-scale studies and therefore used mainly as research tools. Surrogate indices have been derived using OGTT measurements and fasting plasma glucose and insulin, as detailed below.

1.7.3 INDICES DERIVED FROM THE ORAL GLUCOSE TOLERANCE TEST

In view of the limitations of the intravenous methods mentioned above, the OGTT has been studied as a technique to measure insulin sensitivity and secretion. Some of these measures are summarized in Table 1.2.

Table 1.2 – Measures of insulin sensitivity and secretion derived from the oral glucose tolerance test

	Insulin sensitivity	Insulin secretion
Matsuda index (89)	(10,000/square root of [FPG x FPI] x mean glucose x mean insulin)	
Stumvoll index (90)	0.226 – (0.0032 x BMI) – (0.0000645 x Ins120min) – (0.0097 x Gluc90min)	*1st phase:* 1283 + 1.829 x Ins30min – 138.7 x Gluc30min + 3.772 x Ins0min *2nd phase:* 287 + 0.4164 x Ins30min – 26.07 x Gluc30min + 0.9226 x Ins0min
Area-under-curve measures using trapezoidal rules (91, 92)	Various formulae	Various formulae

FPI – fasting plasma insulin in pmol/l, FPG – fasting plasma glucose in mmol/l

1.7.4 INDICES DERIVED FROM FASTING SAMPLES

In order to further simplify estimations of insulin sensitivity and secretion, various groups have derived indices from fasting plasma glucose and insulin values. These are summarised in Table 1.3.

Table 1.3 - Insulin indices derived from fasting parameters

	Insulin sensitivity	**Insulin secretion**
Homeostasis model assessment (HOMA) (93)	FPG (mmol/l) x FPI (mU/l) / 22.5	20 x FPI (mU/l) / (FPG [mmol/l]-3.5)
McAuley's index (94)	exp (2.63 - 0.28 x ln[FPI (microU/ml)] - 0.31 x ln[triglycerides (mmol/l)])	
Quantitative insulin sensitivity check index (QUICKI) (95)	1/[log(FPI in µU/ml) + log(FPG in mg/dl)]	
	FPG / FPI	

FPI – fasting plasma insulin, FPG – fasting plasma glucose

1.7.5 HOMEOSTASIS MODEL ASSESSMENT OF INSULIN RESISTANCE AND SECRETION

The HOMA model was first described by Matthews et al. in 1985 (93). They used measures of insulin secretion and insulin resistance (IR) obtained from hyperglycaemic and euglycaemic clamps and intravenous glucose tolerance tests to correlate with an index derived from fasting plasma glucose and insulin. The model gives an estimate of insulin resistance (normal as 1) and β-cell function (normal as 100%). The HOMA estimate of insulin resistance (HOMA-IR) correlated well with that measured by the euglycaemic clamp in 12 normal subjects (r=0.83, p<0.01), in 11 diabetic subjects (r=0.92, p<0.0001) and in both groups together (r=0.88, p<0.0001). In the same patient groups, the HOMA estimate of β-

cell function correlated well with that measured by the hyperglycaemic clamp (r=0.59, p<0.05 in normal subjects; r=0.71, p<0.02 in diabetic subjects and r= 0.61, p < 0.01 in both groups together).

The physiological basis for the HOMA model is explained as follows [Figure 1.7, (96)]: the relationship between glucose and insulin in the fasting state is a balance between hepatic glucose output and insulin secretion, which is maintained by a feedback loop between the liver and pancreatic β-cells. Hepatic glucose output and uptake are modelled to be dependent on plasma glucose and insulin concentrations (Fig. 1.7 B). Glucose uptake in the adipose tissue and muscle depends on insulin concentration (Fig. 1.7 C and D). The basal glucose output of 0.8 mmol/min is assumed to enter a space of 17 litres. In normal humans, 50% of the basal glucose turnover is in the nervous system which is a glucose-dependent process (Fig. 1.7 E). The glucose uptake by muscle and fat is both glucose and insulin dependent (Fig. 1.7 C and D).

Decreases in β-cell function are modelled by changing the β-cell response to plasma glucose concentrations. Insulin sensitivity is modelled by proportionately decreasing the effect of plasma insulin concentrations at both the liver and the periphery (96). Overall, the glucose turnover in the model remains constant. The HOMA model insulin secretion and insulin sensitivity curves are shown in Figure 1.8.

HOMA-IR has been validated in CKD patients (97, 98) and has been used to estimate IR in ESRF patients on dialysis (99). Crutchlow et al. demonstrated in CKD patients that there is good correlation between insulin sensitivity estimates derived from FSIVGTT and those calculated from fasting glucose and insulin measurements, including HOMA-IR (98). In their validation study, HOMA-IR was significantly correlated to FSIVGTT-derived insulin

sensitivity in 27 patients with CKD stages 2-5, including four patients on haemodialysis (r = -0.51, p=0.006). Furthermore, the HOMA model has been validated in KTRs against gold-standard measures (100, 101). In a previous study by our group, insulin sensitivity measurements from 76 FSIVGTT results in 38 non-diabetic KTRs on tacrolimus-based immunosuppression, were tested against insulin resistance indices calculated from fasting glucose and insulin values (100). Insulin sensitivity correlated with several fasting indices including the HOMA-IR (r = -0.24, p=0.038).

Figure 1.7 - The underlying physiological basis of the HOMA model.

Plasma glucose concentration in the fasting state is regulated by hepatic glucose output, which is insulin dependent (B). Insulin concentration is dependent on β-cell response to glucose (A). Insulin signals glucose uptake in fat and muscle (C and D). Glucose disposal is modelled in brain (E) and kidney (F) as being dependent only on glucose, and in fat and muscle as being dependent on glucose and insulin concentrations [Adapted from Wallace et al. (96)]
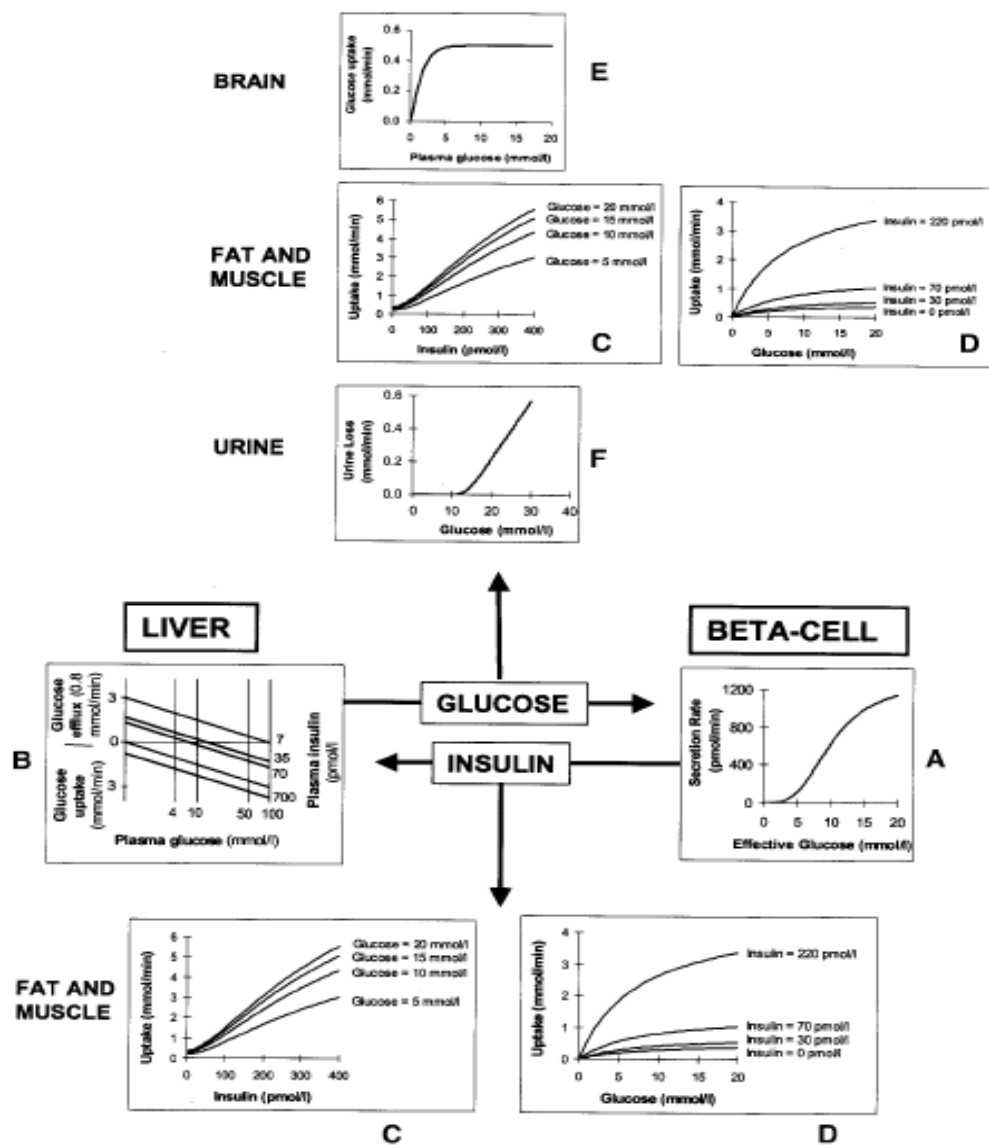
Figure 1.8 – Insulin secretion and sensitivity curves derived from the HOMA model [From Wallace et al. (96)]
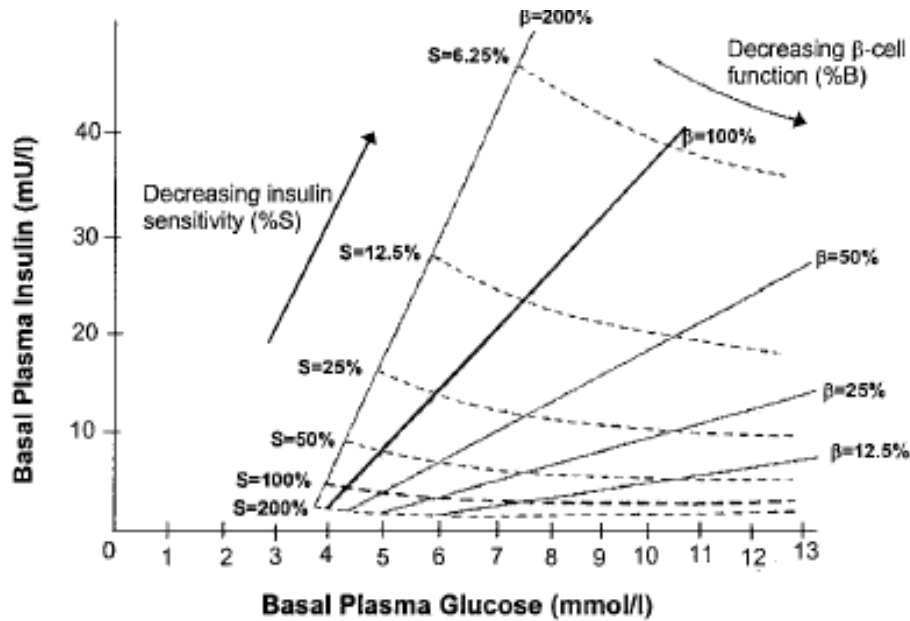
1.7.6 MCAULEY'S INDEX

McAuley et al. have proposed an equation using log-transformed values of fasting insulin and triglycerides to predict insulin sensitivity in population studies (94) (Table 1.3). In an observational study in normoglycaemic volunteers, the euglycaemic insulin clamp method was used to determine insulin sensitivity. They showed that using fasting insulin values in this equation had better sensitivity and specificity than using fasting insulin alone in predicting insulin sensitivity. In a normoglycaemic renal transplant population, our group has previously shown that insulin sensitivity calculated using the McAuley's index correlated well with that measured using an FSIVGTT (r = 0.323, p = 0.005) (100).

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1.7.7 ADVANTAGES AND DISADVANTAGES OF USING SURROGATE MARKERS OF INSULIN SENSITIVITY AND SECRETION

The gold-standard methods for estimating insulin sensitivity/resistance and secretion, i.e. the "clamp" methods and FSIVGTT, are invasive, time-consuming and cumbersome. As a result, they are not suitable for routine use in clinical and epidemiological studies. As we have seen in section 1.6.5, insulin resistance and secretion can be estimated by the HOMA model, which uses fasting glucose and insulin measurements. Therefore, models such as HOMA and the McAuley's index have the advantage of being simple to calculate and easy to use. The HOMA model has been used in large cross-sectional and prospective epidemiological studies in the general population to predict the future development of T2DM (102-105). They can be used to assess response to new treatments for T2DM (106) and to assess the risk of developing T2DM (107). It is particularly advantageous to use the HOMA model in such studies since there is very good correlation between HOMA-IR and insulin sensitivity derived from clamp studies (r>0.8) in normal subjects and T2DM patients with normal kidney function.

Although fasting insulin indices have been validated against gold-standard methods in KTRs, the strength of the correlation is not as strong as in the studies in general population. As mentioned in section 1.6.6 for example, in one study, $r = -0.24$ for the correlation between HOMA-IR and insulin sensitivity from FSIVGTT. Even in patients with CKD, the value of r for the same correlation was only -0.51. The smaller values of r in CKD and KTRs suggest that the two values are related, but may not be suitable for use interchangeably. This in itself may not be an issue if the aim of using these indices is to describe the pathophysiology of hyperglycaemic states in CKD and in KTRs. However, if these indices are used in

longitudinal studies in KTRs, their power to predict future hyperglycaemia may not be as strong as in the general population studies.

1.8 NEW-ONSET DIABETES AFTER KIDNEY TRANSPLANTATION

1.8.1 DEFINITION

There has been an evolution in the definition of NODAT in the past 15 years. The 2003 and 2005 International Guidelines on NODAT recommended the prevailing WHO diagnostic criteria for diagnosing T2DM to be used for diagnosing NODAT (108, 109). At the time of these guidelines, many clinical studies defined NODAT by the need for anti-hyperglycaemic treatment post-transplantation. Clearly, using the treatment threshold for diagnosing NODAT, although easy in retrospective studies, will underestimate the incidence of NODAT by missing less severe cases. A more recent International Consensus Meeting reiterated that the OGTT remains the diagnostic test of choice for diagnosing NODAT (110). Recommendations from this meeting suggested using the American Diabetes Association (ADA) diagnostic criteria for diagnosing DM: symptoms of diabetes plus random plasma glucose >11.1 mmol/L or FPG >7.0 mmol/L or 2-hour PG >11.1 mmol/L during an OGTT or HbA1c >6.5%. One recommendation from this meeting was in fact to change the terminology from NODAT back to post-transplant diabetes mellitus (PTDM). This is in view of the fact that pre-transplant screening to exclude DM is not always possible. Current UK guidelines for the assessment of potential kidney transplant recipients do not mention screening for DM or other dysglycaemias in those not known to have DM (111)

Although HbA1c-based diagnosis of DM is now endorsed by ADA for the general population, caution must be exercised with its use early after kidney transplantation. A normal HbA1c will not exclude a diagnosis of DM in the presence of post-transplantation anaemia or rapidly changing renal allograft function (112).

In summary, NODAT is the development of DM after kidney transplantation in patients with no evidence of DM pre-transplantation. Since pre-transplant DM cannot always be excluded, PTDM may be a better term to use in these cases. NODAT should be diagnosed using the OGTT whenever possible, and ADA diagnostic criteria should be applied to the results of the OGTT.

1.8.2 OUTCOMES

NODAT poses a significant challenge in solid organ transplantation. Its association with reduced patient survival after kidney transplantation has been consistently demonstrated in large epidemiological studies (113, 114) and single-centre studies (115, 116). Data from these studies are summarized in Table 1.4. As seen in Table 1.4, these studies on the impact of NODAT on patient survival have used indirect criteria such as insurance records, medication use or random blood glucose to diagnose diabetes (113, 115, 116). Using indirect criteria to diagnose diabetes has the obvious disadvantage of underestimating the true incidence of NODAT by capturing only the more severe end of the spectrum. Hjelmesaeth et al., using OGTT to diagnose NODAT, found that NODAT was a significant predictor of adverse cardiovascular events but not mortality (117). Therefore, the long-term mortality effect of NODAT diagnosed early after transplantation using fasting glucose criteria is not clearly known.

With regard to macrovascular risk, Cosio et al. studied the effect of post-transplant hyperglycaemia on the occurrence of adverse CV events (118). In a retrospective analysis with a follow-up period of 40 ± 14 months, they found that 12% of KTRs had CV events (cardiac, cerebrovascular, and/or peripheral). Increasing fasting glucose levels at one year post-transplant were significantly related to CV events, independent of other CV risk factors such as older age, CV events pre-transplant, male gender and dyslipidaemia (Figure 1.9). NODAT is also identified as part of the cluster of risk factors for cardiovascular morbidity. For example, in the large multicentre PORT study, NODAT was an independent risk factor for developing coronary heart disease during a follow-up period of three years (HR 1.86, 1.43–2.43) (119). Also, as mentioned above, Hjelmesaeth et al. using OGTT to diagnose NODAT showed that KTRs developing NODAT by three months post-transplant had a three-fold increased risk of adverse CV events as compared to non-diabetic KTRs during an 8-year follow-up (HR 3.27, 95% CI 1.22–8.80, p=0.019).

As seen in the preceding paragraph, adverse CV events occured within a short period of time after the development of NODAT. However, in T2DM patients, with regard to CV risk, it is not clear how long this takes to develop and to what extent it is attributable to co-existing MS, rather than to the raised blood glucose alone. Microvascular complications of T2DM in the general population take >10 years to develop. Therefore in NODAT patients also, it is not clear to what extent the adverse macrovascular complications are due to co-existing traditional risk factors (hypertension, dyslipidaemia, insulin resistance), rather than elevated blood glucose alone. It is also possible that KTRs who developed NODAT in these studies had undiagnosed hyperglycaemia pre-transplantion. This can lead to an increased CV risk in itself. In the general population, IFG and IGT are associated with an increased risk of CV

disease (120). In the study by Cosio et al. mentioned above, KTRs with NODAT at one year had a high incidence of pre-transplant CV events, lending support to this possibility (118).

There are only small reports of KTRs with NODAT developing microvascular complications such as nephropathy or neuropathy (121, 122). However, with a larger proportion of KTRs surviving more than a decade after transplantation in recent years, the incidence of microvascular complications of NODAT could potentially increase.

Figure 1.9 - Cumulative incidence of CV events five years post-transplant in patients classified according to their fasting glycaemia at one year (Data calculated by Kaplan-Meier method, p = 0.003), adapted from Cosio et al. (118)
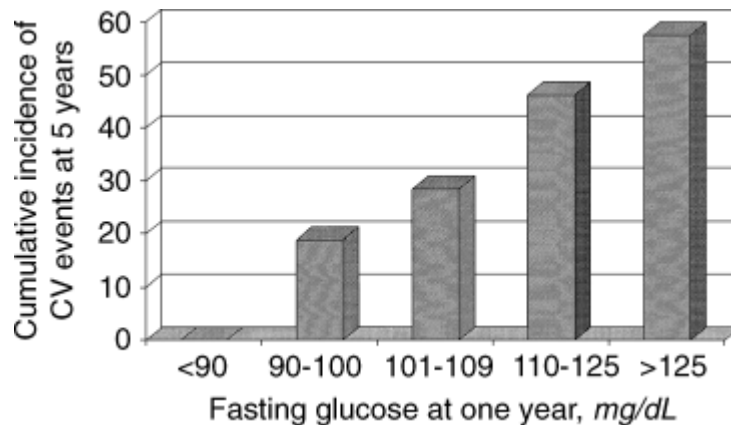
Table 1.4 – A summary of studies reporting on the mortality of kidney transplant recipients with NODAT

Study	Design	Total no. of KTRs	Follow-up	Criteria for NODAT	Mortality with NODAT
Kuo et al. (2010) (123)	OPTN/UNOS database analysis	37,448 with surviving grafts for > one year	Median 548 days (IQR 334-752) after one year	DM recorded on the database post-transplant	HR 1.22 (0.94-1.59) , p=0.10
Hjelmesæth et al. (2006) (117)	Single centre, retrospective	201	8 years	OGTT	HR 1.20 (0.58–2.49), p=0.62
Kasiske et al. (2003) (113)	USRDS registry analysis	11,659	Minimum 3 years	Medicare claims for DM	HR 1.87 (1.60–2.18), p <0.0001
Cosio et al. (2002) (116)	Single centre, retrospective	1,811	Mean 8.3 years	Pharmacological treatment of DM	HR 1.80, (1.35 - 2.41), p=0.001
Revanur et al. (2001) (115)	Single centre, retrospective	939	14 years	HbA1c>6.5% + one of RBG>11, HbA1c>8% or use of hypoglycaemic drugs	10 year survival 49% compared to 75% with no DM

The effect of NODAT on graft survival is not very clear. Although overall graft loss rate is increased due to the higher mortality rate, the effect of NODAT on death-censored graft loss (DCGL) remains unclear. An analysis of the US Renal Data System (USRDS) by Kasiske et al found that NODAT was significantly associated with both an increased overall graft failure (HR 1.63, 1.46–1.84, p<0.0001) and DCGL (HR 1.46, 1.25–1.70, p<0.0001). A different analysis of data from USRDS by Kuo et al. included acute rejection (AR) as a confounding factor (123). They found that NODAT was clearly associated with overall graft failure (HR 1.24, 1.14-1.35) but not with DCGL (HR 1.12, 0.99-1.26). Therefore, it is plausible that the observed association between NODAT and DCGL is due to previous episodes of AR resulting in augmented immunosuppression. The augmented immunosuppression can lead to NODAT and the rejection contributes to DCGL (113).

1.8.3 INCIDENCE OF NODAT

A review by Montori et al. found a varying incidence of NODAT in the literature, ranging from 2% to 50% by the end of first year after transplantation (124). The variety of glycaemic thresholds used to diagnose NODAT and several different immunosuppression regimes used are two factors contributing to the wide variation in the reported incidence of NODAT. In a large single-centre study by Cosio et al., 2078 non-diabetic KTRs transplanted since 1983 were studied. The reported cumulative incidence of NODAT (defined as the need for hypoglycaemic medication) was 7%, 10%, 13%, and 21% at 1, 3, 5, and 10 years after transplantation (125). The highest incidence was found in the first six months after transplantation. All patients received ciclosporin and none received tacrolimus in this study.

Woodward et al. analysed the USRDS and the United Network of Organ Sharing (UNOS) databases to determine the incidence of NODAT, which was defined to have occurred on the

date of the second Medicare claim for DM (126). Again, they found the incidence of NODAT to be highest in the first year after transplantation in ciclosporin treated patients. However, the incidence of NODAT was higher than baseline even after the first year in tacrolimus treated patients (Figure 1.10). This study strongly suggested the critical role of receiving a kidney transplant and the subsequent immunosuppressive drugs in increasing the incidence of DM over and above the background rate in waitlisted patients.

Figure 1.10 - Incidence of diabetes before and after transplant by type of calcineurin inhibitor (From Woodward et al. (126)). All pre-transplant patients analysed were on the waiting list for a deceased donor transplant (FK506 – tacrolimus).
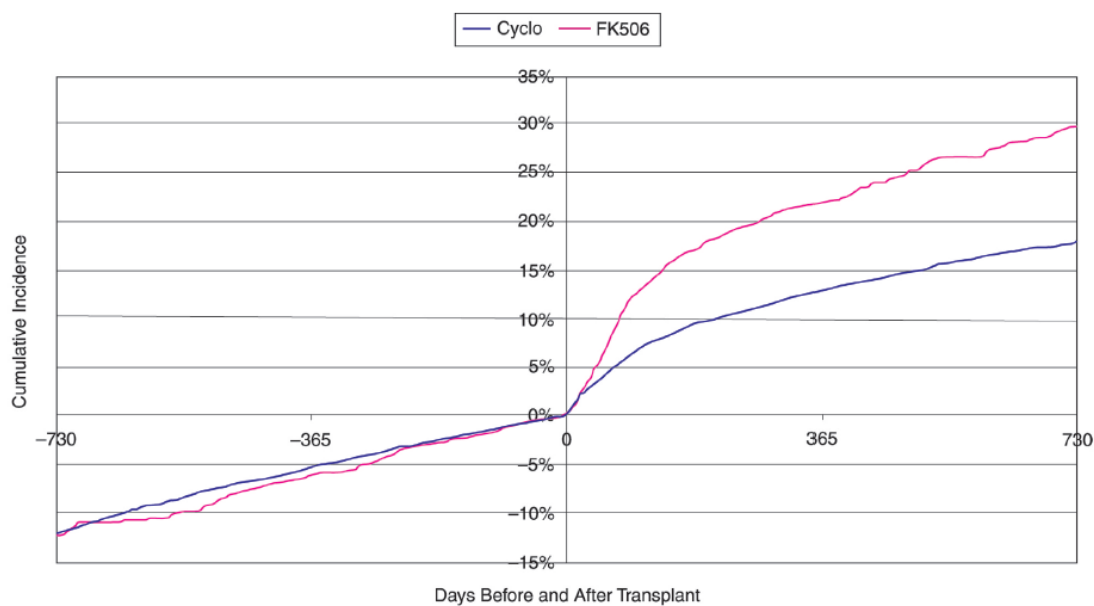
The review of NODAT incidence by Montori et al. included studies conducted in the 1990s and since that era, there is some evidence that the incidence of NODAT may have decreased in more recent years. For example, Valderhaug et al. compared the incidences of NODAT

diagnosed using OGTT at three months post-transplantation in two different cohorts - one transplanted in 1995-1996 and the other in 2004-2005 (127). They found that the incidence of NODAT was significantly lower in the new cohort (13%) compared to that in the historical cohort (20%). This finding was despite the fact that patients in the new cohort were older and had a higher BMI. Even on multivariate analysis taking into account the differences in demographics and immunosuppression, patients in the new cohort had significantly lower odds of developing NODAT (odds ratio 0.42, 95% CI 0.23 – 0.77, p<0.005).

In Cardiff, a study conducted by Ravindran et al. revealed a cumulative incidence of NODAT of 20% by the end of one year and 27% by the end of four years amongst patients receiving a transplant between 1996 and 2001 (128). The incidence was higher for those patients receiving tacrolimus (34%) compared to those receiving ciclosporin (17%). The incidence of NODAT in more recent years in Cardiff is not known.

1.8.4 RISK FACTORS FOR NODAT

Similar to T2DM, both modifiable and non-modifiable factors play a role in the development of NODAT. Certain factors are unique to the setting of transplantation and recognition of these factors is essential to risk-stratify individual patients in order to possibly tailor immunosuppressive therapy (129). Table 1.5 shows a summary of the risk factors for NODAT.

Table 1.5 – Risk factors for NODAT

Transplant-specific factors	Risk factors common with T2DM
Immunosuppression (tacrolimus, ciclosporin, sirolimus, corticosteroids)	Increasing age
	Family history of T2DM
Cytomegalovirus infection	Ethnicity (African, Asian, Hispanic)
Acute rejection	Obesity
Hepatitis C virus infection	Metabolic syndrome
	High serum triglyceride level
General factors	Single nucleotide polymorphisms of certain genes
Autosomic-dominant polycystic kidney disease	
Hypomagnesaemia	

1.8.4.1 NON-MODIFIABLE FACTORS

I. AGE

Several epidemiological and clinical studies have demonstrated that increasing age is a strong risk factor for NODAT, just as it is for T2DM (113, 125, 130). Since the pathophysiology of NODAT has some similarities to that of T2DM, it is not surprising that older patients are more at risk of developing NODAT.

II. ETHNICITY

African-American and Hispanic recipients are at a higher risk of developing NODAT. A US study based on the USRDS database by Kasiske et al. reported a relative risk of 1.68 and 1.35 respectively for these two races compared with white recipients (113). A more recent analysis

of the OPTN/UNOS database from the US also found that African-American race to be associated with the development of NODAT (RR 1.32) (131).

III. CAUSE OF ESRF

Certain causes of ESRF have been associated with an increased risk of NODAT. For example, patients with autosomal dominant polycystic kidney disease (ADPKD) have been shown to be at an increased risk of developing NODAT (132-134). The mechanism of this association is not clear, although one small study found that adults with ADPKD and normal renal function had a lower insulin sensitivity compared to age- and gender-matched healthy controls (135). In the large US study by Kasiske et al., even after correcting for age and race, glomerulonephritis (GN) was associated with a lower relative risk of developing NODAT (0.80, p<0.0001) compared with other causes of ESRF (113). NODAT was defined using Medicare claims. Since this was a registry-based study, there was no detail on how many of the subjects with GN had been treated with steroid pre-transplantation. Also, the authors acknowledged that other pre-transplant risk factors and the definition used for NODAT may have had a bearing on the results.

IV. GENETIC POLYMORPHISMS

With the advent of genetic profiling and identification of various genetic polymorphisms as risk factors for T2DM, similar studies have been undertaken in KTRs to look for risk factors for NODAT. For example, as seen in section 1.5.2, genetic variants in the gene encoding for TCF7L2 are known to impair insulin secretion and incretin effects (62). The rs7903146 polymorphism of the *TCF7L2* gene has shown to be associated with an increased risk of NODAT in both European (136) and Korean (137) KTRs. Nicoletto et al. found that the 276G/T adiponectin gene polymorphism was associated with NODAT in a population of

Caucasian KTRs in Brazil (138). Other studies have noted that variations in the *KCNQ1* gene and the mitochondrial haplogroup H were associated with increased risk for NODAT among tacrolimus-treated KTRs (139, 140). One recent Korean study investigated 18 single nucleotide polymorphisms (SNPs) located within the 10 genes of interleukins (IL) or their receptors and found that 10 SNPs were associated with an increased of NODAT, suggesting that inflammation of β-cells may have a role to play in the pathogenesis of NODAT (141). We cannot draw any firm conclusions from this study as the total number of subjects was 306, which is a small sample size for a genome-wide association study.

V. FAMILY HISTORY OF T2DM

The risk of developing NODAT conferred by a family history of T2DM is not very clear. Many studies reporting on the incidence and risk factors for NODAT have not collected data on the family history of T2DM. Amongst those studies which have reported family history data, the results are conflicting. Some studies have shown an association between family history of T2DM and NODAT (130), whereas other studies have not shown this association (142, 143).

In summary, recent genetic studies strongly suggest that the pathogenesis of NODAT may be closely linked to that of T2DM, and consequently, a family history of T2DM would be an important factor to be considered in studies on NODAT.

1.8.4.2 MODIFIABLE FACTORS

I. OBESITY

Obesity, measured either as BMI or weight, is associated with an increased risk of NODAT. This has been observed consistently in a number of studies (113, 116, 144). Kasiske et al.

found in their analysis of USRDS data that NODAT was significantly more common in those with a BMI > 30 kg/m² compared to those with a BMI < 30 kg/m² (RR 1.73, p<0.001) (113). Similarly, Joss et al. in Glasgow showed in a retrospective analysis that higher patient weight is a risk factor for developing NODAT (144). On the other hand, the association between weight gain post-transplantation and the risk of developing NODAT has been contrasting in different studies. Two large studies by Cosio et al. and Marrero et al. found an association between NODAT and pre-transplant weight / BMI, but not the weight-gain in the first year post-transplant (125, 145). In contrast, two other studies did find an association between weight-gain and the development of NODAT (146, 147). It is possible that in some transplant centres, KTRs who are identified as high risk for NODAT have their steroid dose reduced or lifestyle measures instituted early to account for the lack of weight-gain. However, other strong factors such as CNIs and increased age may overpower the weight-gain aspect and still lead to the development of NODAT in these patients.

II. METABOLIC SYNDROME

MS is a cluster of cardiovascular risk factors (central obesity, hypertension, dyslipidaemia, and glucose metabolism abnormalities) which increases the risk of developing T2DM and cardiovascular morbidity and mortality (148-150). In the general population, MS has been associated with the development of new T2DM (151). Bayer et al. reported that in a US population of potential KTRs, MS was common pre-transplantation (57.2 %) (152). NODAT developed in 31.4% of recipients one year post-transplant. KTRs with MS were more likely to develop NODAT compared with recipients without MS (34.4% vs. 27.4%, p=0.057). In the post-transplant setting, MS diagnosed at 12 months post-transplantation was a risk factor for developing NODAT in a retrospective study by Porrini et al. (153). There are no studies

linking MS diagnosed >12 months after renal transplantation and subsequent glucose intolerance.

III. CYTOMEGALOVIRUS INFECTION

Hjelmesaeth et al. noted in a single-centre report that asymptomatic cytomegalovirus infection was associated with the development of NODAT (154); direct virus-induced damage to pancreatic β-cells was suggested as one of the mechanisms of this association (155). No other group has reported this observation.

1.8.4.3 FACTORS UNIQUE TO THE TRANSPLANT SETTING

I. CALCINEURIN INHIBITORS

Immunosuppression is thought to play an important role in the causation of NODAT. Indeed, the type of immunosuppression explained 74% of the variability in the incidence of NODAT in a systematic review by Montori et al. (124). Several registry-based studies, randomised trials and meta-analysis have shown an increased risk of NODAT with the CNI tacrolimus compared to ciclosporin (53, 113, 156).

In a meta-analysis by Heisel et al., 35 publications reporting the incidence of NODAT in KTRs treated with either tacrolimus or ciclosporin were analysed (53). All studies were conducted prior to 2003. The mean incidence of NODAT in 25 tacrolimus-based studies was 15.4%, compared with 9.8% across all 21 cicloporin-based studies. The type of concomitant immunosuppressants did not appear to have a bearing on the incidence of NODAT. Furthermore, when seven prospective, randomized studies that directly compared tacrolimus and ciclosporin-based regimens were analysed, the average NODAT rate was 11.5% for tacrolimus-treated patients and 4.7% for ciclosporin-treated patients.

More recently, the open-label, randomized, multi-centre DIRECT study (Diabetes Incidence after Renal Transplantation: Neoral C2 Monitoring Versus Tacrolimus, N=567) specifically compared the incidence of NODAT in *de novo* KTRs treated with tacrolimus or ciclosporin (156). The composite primary safety end-point of NODAT or impaired fasting glucose at six months occurred in 73 ciclosporin-treated patients (26.0%) and 96 tacrolimus-treated patients (33.6%, p = 0.046). There was no difference in short-term graft or patient outcomes between tacrolimus and ciclosporin groups. In the ELITE-Symphony study too, which compared low-dose tacrolimus with standard-dose ciclosporin, low-dose ciclosporin, and low-dose sirolimus, the incidence of NODAT was highest in the tacrolimus arm (10.6%) and lowest in the low-dose ciclosporin arm (4.7%) (157).

In an analysis of the OPTN/UNOS database of 15,309 KTRs, Shah et al. reported that the relative risk of developing NODAT was 1.5 with tacrolimus use compared to non-use (131). In a different analysis of the same database, a dose-response relationship was seen between increasing tacrolimus and steroid doses and NODAT (158). Interestingly, the cumulative incidence of NODAT in KTRs treated with very low doses of tacrolimus (<0.12mg/kg/day) was still higher than in those treated with ciclosporin (Figure 1.11). For patients treated with ciclosporin, the measured dosage of ciclosporin, the dosage of steroids at 30 days, and their interaction, were not significantly associated with NODAT.

In summary, there is evidence from RCTs, meta-analysis and multivariate analyses of large registry data to show that the type of immunosuppression used affects the incidence of NODAT, and that the incidence with tacrolimus is higher than that with ciclosporin.

Figure 1.11 - Kaplan-Meier estimates of the incidence of NODAT in patients stratified according to tacrolimus and steroid dosage at 30 days after transplantation, with ciclosporin (CsA) as a reference [Taken from Burroughs et al. (158)]
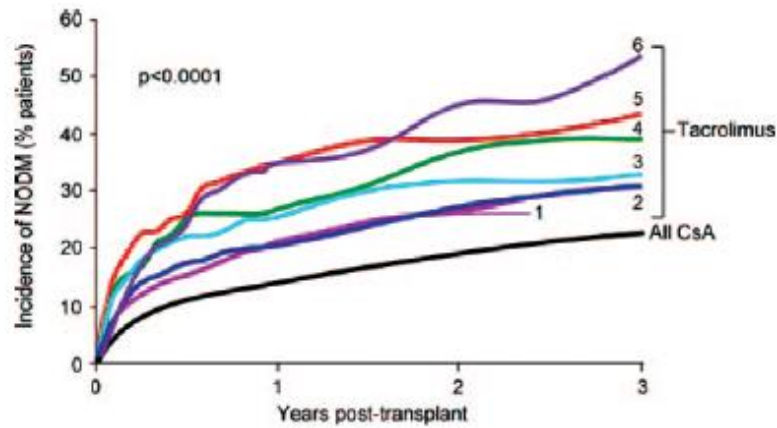
Group	Steroid dose (mg/kg/day)	Tacrolimus dose (mg/kg/day)	Incidence of NODM (%)
6 (n=81)	>0.75	>0.23	54%
5 (n=219)	>0.75	0.12-0.23	43%
4 (n=159)	>0.75	<0.12	39%
3 (n=273)	<0.75	>0.23	33%
2 (n=966)	<0.75	0.12-0.23	31%
1 (n=1,197)	<0.75	<0.12	31%
All CsA (n=5,943)	0.0-1.23	0.10-25.0 (CsA)	23%

II. SWITCHING BETWEEN CNIS

Data is conflicting on the issue of whether switching between the two CNIs affects glucose metabolism. One observational study in the USA analysed a cohort of 704 non-diabetic KTRs in whom a medically indicated switch was performed from ciclosporin to tacrolimus (for rejection, ciclosporin toxicity or unstable blood levels) (159). Statistical analyses took into account the duration of therapies before and after the switch. The adjusted 5-year NODAT-free survival of 87.4% in those 171 patients who converted from ciclosporin to tacrolimus was similar to those 533 who continued on ciclosporin (91.4%, p = 0.90). Age, BMI and a

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previously high plasma glucose level were found to be significant risk factors for NODAT. One limitation of this study was that the subjects who switched to tacrolimus were younger and had lower BMIs than those who did not switch.

In a prospective, cross-over, mechanistic study, 30 non-diabetic stable KTRs were converted from ciclosporin to tacrolimus ten years after transplantation (160). Median ciclosporin level was 120 µg/l before conversion and median tacrolimus level was 6.4 µg/l after conversion. Using IVGTT, it was shown that switching from ciclosporin to tacrolimus did not adversely affect stimulated insulin secretion measured before and one month after stable tacrolimus levels post-conversion.

On the other hand, one small retrospective study reported beneficial effects of switching from tacrolimus to ciclosporin in 34 KTRs with NODAT (161). One year after converting from tacrolimus to ciclosporin, there was a decrease in fasting plasma glucose and HbA1c in these patients compared to the 20 who continued on tacrolimus. There was also remission of NODAT (FPG <7 mmol/l with no anti-diabetic medication) in 42% of patients who did convert from tacrolimus to ciclosporin. How long after transplantation NODAT developed and when the switch was made is not clear from the article.

In a small controlled study in KTRs diagnosed with NODAT, 8 of the 28 patients were converted from tacrolimus to ciclosporin after a mean of 11 months from transplantation (162). All five patients in the study group who were on anti-diabetic medication prior to the switch were able to either stop (n=3) or reduce the doses (n=2), in comparison to the control group in whom no such beneficial effects were seen.

No adverse effects of converting from one CNI to the other were reported in any of the studies mentioned above.

In summary, some beneficial effects of converting from tacrolimus to ciclosporin on glucose metabolism have been reported by one controlled study and some retrospective observational studies. However, caution has to be exercised in applying this to clinical practice until there is more robust evidence from randomised trial data. In fact, a recent expert committee meeting recommended caution in immunosuppressant adjustments to improve glucose metabolism after the development of NODAT (110). Immunological risk for rejection also has to be accounted for, and the recommendation was therefore to use immunosuppression regimens shown to have the best outcome for patient and graft survival.

III. SIROLIMUS

The mammalian target of rapamycin (mTOR) inhibitor sirolimus has been associated with an increased risk of NODAT (163). In one prospective study, two groups of patients were studied: one group switching from ciclosporin to sirolimus for a diagnosis of chronic allograft nephropathy (n=32), and the second group continuing sirolimus alone after stopping tacrolimus at three months post-transplant (n=15) (164). Switching to a sirolimus alone regimen was associated with a 30% increase in the incidence of impaired glucose tolerance with four patients developing NODAT. This regimen was also associated with a worsening of insulin resistance and an inappropriately low insulin response, both of which correlated with high serum triglyceride levels.

IV. CORTICOSTEROIDS

High-dose corticosteroid use in the first year after transplantation to prevent acute rejection has traditionally been considered to be an important cause of early glucose dysregulation. However, the diabetogenecity of steroids seems to be dose-dependent (130, 165). In a study by Hjelmesaeth et al., each 1 mg reduction in prednisolone dose decreased the 2-hour plasma glucose level by an estimated 0.12 mmol/l (165). Tapering of prednisolone dose improved glucose tolerance during the first year after kidney transplantation.

As seen above, in a UNOS registry study of 8839 non-diabetic KTRs, higher steroid doses early after transplantation increased the incidence of NODAT in tacrolimus-treated patients, but not in ciclosporin-treated patients (158).

Over the last few years, efforts have been made to develop regimens which aim to minimise steroid exposure. For example, Woodle et al. compared outcomes in KTRs treated with chronic low-dose steroid therapy (5 mg/day from six months, n=195) with those with early steroid withdrawal (within seven days post-transplant, n=191) (166, 167). No differences were observed at five years in the proportion of patients experiencing death-censored graft loss or death. The 5-year incidence of NODAT in those KTRs treated with early steroid withdrawal (39.4%) was similar to those who continued 5 mg/day of prednisolone (39.3%). Also, the proportion of patients requiring treatment for NODAT was similar between the two groups (22.5% vs. 21.5%). The results of this study suggest that the use of low-dose steroids (5 mg/day from month six) combined with tacrolimus and mycophenolate mofetil (MMF) maintenance therapy has a limited effect on NODAT risk compared to early steroid withdrawal.

A systematic review of RCTs of steroid minimisation (steroids completely withdrawn randomly between 3 and 6 months post-transplantation) noted that there was a trend towards a lower incidence of NODAT in KTRs in whom steroid therapy was withdrawn (three RCTs, 656 participants, RR 0.58, 95% CI 0.31–1.09, p=0.089) (168). Since only three RCTs in this review reported on NODAT, the reviewers concluded that a larger sample size may have yielded a significant result. They also concluded that the diabetogenic effect of CNIs may outweigh the benefit of steroid withdrawal (168).

1.9 PREDICTION OF NODAT

Since NODAT is a serious complication after renal transplantation, it is important to identify risk factors as early as possible, preferably prior to transplantation. Effective preventative measures can be implemented post-transplantation in the form of lifestyle intervention (169). Pre-transplant risk factors have been used in attempts to predict the development of NODAT in KTRs (170, 171). From a retrospective analysis of 316 KTRs, Chakkera et al. developed a scoring system using pre-transplant characteristics to predict the development of NODAT (170). A summary score of 0–7, calculated using the dichotomized variables of recipient age (>50 years), family history of T2DM, BMI (>30), fasting glucose (>5.6 mmol/l) and triglycerides (>2.26 mmol/l), use of gout medicine, and predicted use of corticosteroids post-transplant (non-transplant indications or immunologic indications) predicted the occurrence of NODAT at one year post-transplant. The incidence of NODAT ranged from 7% with a score of zero, to 56% with a score of ≥4. The area under the ROC curve for predicting NODAT using the summary score was 0.70. The authors went on to study the score in a new validation population of 474 KTRs (172). However, in the validation study, the ROC area under the curve for the summary score was lower at 0.65. Also, using the summary score

model with the validation cohort showed evidence of lack of fit (p = 0.05). However, using the same seven variables in a predictive equation performed better with the area under the ROC curve of 0.70 (95% CI 0.659–0.724).

Rodrigo et al. have studied the prediction of NODAT beyond the first year after transplantation (173). In a cohort of 191 KTRs who were non-diabetic at one year post-transplantation, 41 patients developed NODAT during further follow-up. They used two DM prediction scores: 1. San Antonio Diabetes Prediction Model (SADPM) (174) and 2. Framingham Offspring Study-Diabetes Mellitus (FOSDM) (175). Areas under the ROC curve for FOSDM and SADPM scores to predict NODAT were 0.756 and 0.807 (p<0.001) respectively. FOSDM and SADPM scores above the 75th percentile were associated with NODAT (hazard ratio 5.07 and 8.18, respectively, p<0.001).

In the general population, insulin resistance and secretion values estimated by HOMA have been used to predict the future development of T2DM (102-104). While two studies found that HOMA was useful in predicting the development of T2DM (102, 103), one Korean study questioned the utility of HOMA in predicting DM (104). To date, no studies aiming to predict NODAT have included insulin resistance or secretion indices in their models.

1.10 PHARMACOLOGICAL PREVENTION AND TREATMENT OPTIONS FOR POST-TRANSPLANT HYPERGLYCAEMIA AND NODAT

International Consensus Guidelines on NODAT were developed by an expert panel in 2003 (108), which were updated and published in 2005 (109). These guidelines, which were endorsed by the International Diabetes Federation (IDF), aimed to reduce cardiovascular risk

of patients undergoing transplantation through earlier recognition and management of NODAT. The recommendation was to diagnose NODAT and IGT based on the WHO classification of T2DM. Figure 1.12 summarises the recommendation from the 2005 update. The guidelines emphasised the need for regular monitoring of fasting blood glucose post-transplantation in order to diagnose NODAT early. Once a diagnosis of NODAT is made, they suggested checking HbA1c levels every three months to determine the need for intervention, and chronic hyperglycaemia to be managed on an individual basis by setting target blood glucose controls and taking a "treat-to-target" approach. Lifestyle modification plays a key role in the management of hyperglycaemia as in T2DM. The guidelines suggested pharmacological therapy as for T2DM, with caveats attached regarding metformin and lactic acidosis.

The 2005 guidelines also acknowledged the lack of randomised trials of pharmacotherapy for the prevention or treatment of NODAT. Furthermore, since the publication of these guidelines, newer classes of anti-diabetic medication have become available in the form of meglitinides, glucagon-like peptide-1 (GLP-1) agonists, dipeptidyl peptidase-4 (DPP-4) inhibitors and sodium-glucose co-transporter-2 (SGLT2) inhibitors. The role of these newer agents in the management of NODAT is not clearly known. Table 1.6 lists the currently available anti-diabetic agents and their potential advantages and disadvantages with respect to transplant recipients.

Figure 1.12 – Blood glucose control recommendations from the 2005 International Consensus Guidelines on NODAT
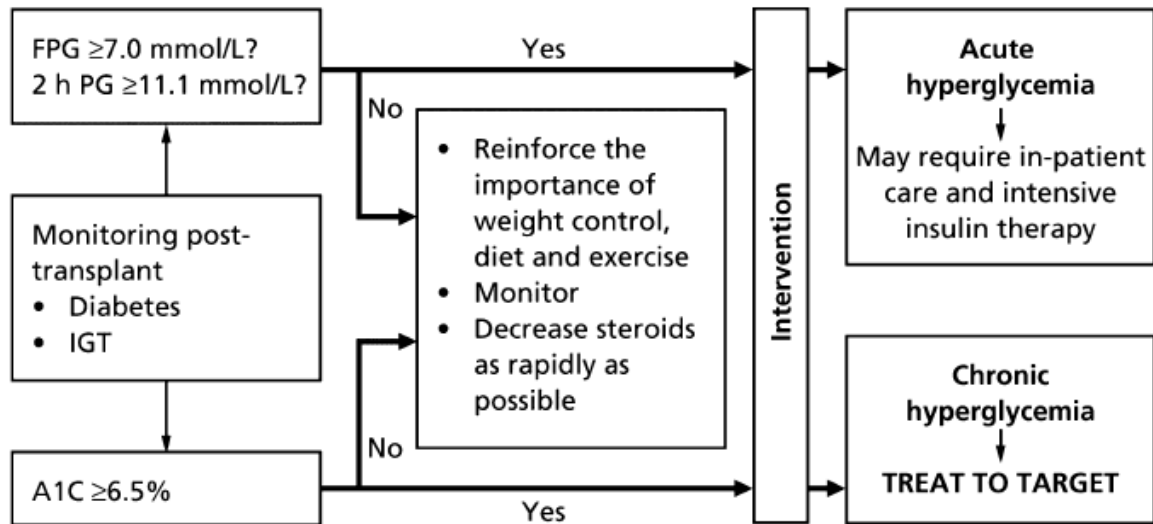
Table 1.6 – Summary of anti-diabetic medications

Agents	Mechanism of action	Advantages	Disadvantages	Interaction with IS drugs
Sulphonylureas	Stimulation of insulin secretion	Well established safety and efficacy in T2DM	Weight gain, accumulation with low GFR and hypoglycaemic risk	No
Biguanides	Enhancement of sensitivity to insulin	Well established safety and efficacy in T2DM, no hypoglycaemia or weight gain	Risk of lactic acidosis with low GFR	No
Thiozolidinediones	Increase in insulin sensitivity	Less risk of hypoglycaemia	Weight gain, oedema, fracture risk, cardiovascular risk	Pioglitazone with CNIs
Meglitinides	Stimulation of insulin secretion		Hypoglycaemia, weight gain, no long-term studies	Not known
GLP-1 agonists	Stimulation of insulin secretion, reduction in glucagon secretion, delayed gastric emptying	Less risk of hypoglycaemia, no weight gain	Lack of long-term safety data	Not known
DPP-4 inhibitors	Increase in incretin levels	No weight gain, some agents can be used with low GFR	Concern regarding pancreatitis	No
Insulin		Efficacy	Weight gain, hypoglycaemia	No

IS - immunosuppression

More recently, there have been a few studies examining the effects of the newer anti-diabetic medication for the treatment of NODAT. In one pilot study involving 15 stable KTRs with NODAT, the DPP-4 inhibitor sitagliptin was shown not to affect tacrolimus or sirolimus levels over a three month period (176). There was no change in eGFR, and HbA1c improved from a baseline mean of 7.2% to 6.7% (p=0.002).

In another phase 2 trial, Haidinger et al studied the safety and efficacy of the DPP-4 inhibitor vildagliptin with a randomised, placebo-controlled, double-blind design (177). They randomised 17 stable KTRs with NODAT to receive vildagliptin and 16 to receive placebo. The primary end-point was the intraindividual difference in the 2-hour plasma glucose level from baseline to three months. This difference was a mean of 4.1 mmol/l for the vildagliptin group compared to 0.3 mmol/l for the placebo group (p<0.01). There were no serious adverse events observed. Estimated GFR and blood CNI levels were not affected by the intervention.

Another study investigated the use of repaglinide in KTRs with NODAT (178). In a retrospective observational study, Turk et al. compared results from 23 KTRs with NODAT who were treated with repaglinide with a control group of 19 patients treated with rosiglitazone. Fourteen of the 23 patients treated with repaglinide for six months had a fall in HbA1c level from a mean of 7.6 ± 0.6% to 5.8 ± 0.6% (p<0.05). In nine patients hyperglycaemia persisted, and they were switched to insulin treatment (HbA1c 8.5 ± 2.9% at baseline to 7.4 ± 2.2% in six months). There were no significant changes in blood CNI levels or S. creatinine concentration during the treatment period. Results were similar to those obtained by using rosiglitazone in the control group.

Werzowa et al. have studied the use of vildagliptin and pioglitazone in KTRs diagnosed with IGT, in a 3-month, double-blind, placebo-controlled, randomized clinical study (179).

Seventeen patients each were treated with the study drugs and 17 control patients were given placebo. Compared with baseline, 2-hour plasma glucose levels were significantly reduced after three months in the vildagliptin and pioglitazone groups. FPG was only significantly reduced in the pioglitazone group. HbA1c levels decreased significantly in both treatment groups (vildagliptin 5.7±0.3 to 5.6±0.3, p=0.046 and pioglitazone 6.2±0.5 to 6.0±0.4, p=0.03). In the placebo group, no changes in the metabolic parameters were observed. There were no significant changes in blood CNI levels or S. creatinine concentration during the treatment period.

In an interesting study, Hecking et al. tested the question whether basal insulin therapy during the immediate post-transplantation period would be an efficacious strategy to control post-transplant hyperglycaemia in previously non-diabetic patients (180). The primary end-point in this study was HbA1c at three months and hypoglycaemic events as the secondary end-point for safety. They randomly assigned 50 KTRs to receive either isophane insulin if their evening blood glucose level was >7.8 mmol/l, or receive standard treatment (short-acting insulin ± oral hypoglycaemic agents) for blood glucose levels between 10-13.9 mmol/l. Although HbA1c increased in both groups from baseline to 3-6 months, the mean HbA1c in the study group was significantly lower than that in the standard treatment group at three months (5.7% vs. 6.2%) and six months (5.8% vs. 6.3%) post-transplantation. By 12 months post-transplantation, none of the patients in the study group were on any anti-diabetic medication. The odds of being diabetic (defined by an OGTT) by 12 months was lower in the treatment group (OR 0.27, CI 0.08-0.95). Despite the limitation associated with using HbA1c at three months as an end-point (112), this study has highlighted the potential importance of "β-cell rest" during times of stress in order to reduce the longer term incidence of NODAT.

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In conclusion, the use of established anti-diabetic agents for post-transplant hyperglycaemia and NODAT has been extrapolated from their use in T2DM. Although newer agents have been shown to be safe and efficacious in small studies, larger and longer-term studies are still warranted.

1.11 SUMMARY

Gold-standard measures of insulin sensitivity and secretion used to elucidate the pathophysiology of post-transplant hyperglycaemia are invasive, time-consuming and cumbersome. As a result, they are not suitable for routine clinical use in prediction models for NODAT or to determine the primary pathophysiological defect of glucose dysregulation in clinical studies. As we have seen in section 1.7.5, insulin resistance and secretion can be estimated by the HOMA model, which uses single FPG and FPI measurements. This model has been validated against gold-standard techniques for measuring insulin resistance and secretion (93). McAuley's index is another measure of insulin sensitivity that closely correlates with that obtained from gold-standard techniques (94). These indices are simple and easy to calculate and hence are useful to estimate insulin resistance in clinical studies. HOMA has been used in general population studies to predict the future development of T2DM with conflicting results. However, the utility of fasting insulin indices in predicting NODAT is unknown in the renal transplant population.

The OGTT has many advantages over fasting plasma glucose for diagnosing glucose metabolism abnormalities as it not only accurately identifies subjects with DM but also identifies those with IFG and IGT. It is also established as a sensitive tool to detect NODAT and glucose intolerance in KTRs (181, 182). Moreover, the 2-hour glucose level of the OGTT

is more closely linked to all-cause and cardiovascular mortality than a fasting glucose level in KTRs (183). In the general population, abnormal glucose tolerance determined by OGTT is a risk factor for the future development of T2DM (184, 185).

Although the surge in the incidence of NODAT occurs in the first year after transplantation, there is still an increasing incidence of DM after the first year which is above the background rate of DM (126). As we have seen earlier that the pathophysiology of NODAT has some features in common with T2DM, it is plausible that there is a continuum of glucose tolerance abnormalities before progression to NODAT. However, there are no studies examining the progression of glucose intolerance diagnosed late after transplantation in KTRs. Also, there are no studies linking MS diagnosed >12 months after renal transplantation and its effect on subsequent glucose intolerance.

The reported incidence of NODAT varies widely in the literature. This variation in the incidence is presumably due to varying definitions used, differences in immunosuppression regimens and different post-transplant periods studied. Longitudinal studies have reported an increasing incidence of NODAT up to 10 years after transplantation (125). As seen in section 1.8.3, this observation has been made in Cardiff too, in a study by Ravindran et al. (128).

Since the study by Ravindran for the period of 1997-2001, various changes have taken place in the immunosuppression policies of CTU. Specifically, induction immunosuppression therapy has been introduced in the form of anti-thymocyte globulin (ATG) for DCD transplants and the interleukin-2 receptor antagonist (IL2RA) basiliximab for DBD transplants. In addition, changes to maintenance immunosuppression therapy have also been implemented: Ciclosporin therapy for de-novo transplants has been discontinued (all patients now receive tacrolimus), the starting dose of tacrolimus has progressively decreased and

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steroid therapy is aimed to be discontinued as early as possible and by three months in view of lower acute rejection rates. These changes offer the opportunity to study the effects of differences in immunosuppressive regimes on the incidence of NODAT in our unit.

Finally, the impact of diagnosing NODAT early after renal transplantation on patient outcomes needs to be examined thoroughly. That is, previous studies have used indirect methods to identify only the severe end of the spectrum of NODAT. The effect of NODAT not needing pharmacological treatment on patient and graft outcomes remains to be established.

1.12 HYPOTHESES AND AIMS OF STUDIES PRESENTED IN THE THESIS

1.12.1 CENTRAL HYPOTHESES

1. NODAT is distinct from T2DM and is due to factors unique to the transplant setting, of which the predominant factor is the use of specific immunosuppressive agents in the early post-transplant period, and traditional risk factors for T2DM are the more significant factors late after transplantation.

2. Fasting insulin indices can be used to describe the pathophysiology of NODAT and they can predict the occurrence of NODAT.

3. With modern immunosuppression regimens, the incidence of NODAT is lower compared with older regimens.

4. Early-diagnosed NODAT exerts a negative impact on patient outcomes, and this is due to association with the traditional CV risk factors.

1.12.2 Aims

a) To describe the pathophysiology of NODAT in KTRs using fasting insulin indices.

b) To determine whether these indices calculated pre-transplantation and early post-transplantation predict the development of NODAT in KTRs.

c) Using OGTT, to investigate the progression of glucose tolerance abnormalities developing late after transplantation.

d) To identify the risk factors associated with the development of early (<12 months) and late (>12 months) NODAT.

e) To examine the association between the metabolic syndrome identified late after transplantation and glucose intolerance.

f) To determine the incidence of glucose metabolism abnormalities in a recent era of transplantation.

g) To determine the effect of early-onset NODAT on long-term patient and graft survival.

Material presented in this thesis comprises work carried out over a period of two years in the Cardiff Transplant Unit at UHW. The aims and hypotheses listed above were tested in three different cohorts of patients, which are described in detail in the following chapter.

CHAPTER 2: METHODS AND DESCRIPTION OF COHORTS

2.1 CIC VS. TAC STUDY COHORT

2.1.1 STUDY DESIGN AND POPULATION

This was a single centre retrospective cohort study. Patients were derived from a larger group of 150 patients who received a deceased donor renal allograft as part of a randomized trial of tacrolimus *versus* ciclosporin undertaken between 1996 and 2001 at UHW (Cic vs. Tac study) (186). Patients were assigned randomly on a 1:1 basis to receive either tacrolimus- or ciclosporin-based triple therapy immunosuppression. Details of the immunosuppression regimen are described in a later section.

Inclusion criteria for the current study were as follows: (a) Age at transplant 18-80 years (b) No history of T2DM and fasting plasma glucose <7.0 mmol/l or random plasma glucose <11.0 mmol/l on at least two occasions in the year before transplantation. Based on these criteria, for the purpose of the current study, 118 patients were derived from the original Cic vs. Tac study. The remaining 32 patients were excluded as they did not meet the DM exclusion criteria as described above. The study obtained approval from the South East Wales Research Ethics Committee and the R & D Unit of UHW.

2.1.2 INSULIN RESISTANCE INDICES

As part of the original Cic vs. Tac study, fasting levels of plasma glucose, insulin and triglycerides were measured pre-transplantation. Post-transplantation, these parameters were measured in the transplant clinic after a 12 hour fast, along with other routine blood tests. For the current study, using these fasting values, the following indices were calculated at baseline (pre-transplantation), three months and twelve months after transplantation -

- IR-HOMA (homeostasis model assessment of insulin resistance) (93) = FPG (mmol/l) x FPI (mU/l) / 22.5

- McAuley's index (94) =

  exp (2.63 - 0.28 ln[FPI (microunits/ml)] - 0.31 x ln[triglycerides (mmol/l)])

- HOMA secretion index (HOMAsec) (93) = 20 x FPI (mU/l) / (FPG [mmol/l]-3.5)

The physiological basis for the above models and a review of them as measures of insulin resistance and secretion are given in section 1.7.8.

2.1.3 DEFINITIONS

Fasting glucose tolerance was determined according to the WHO classification based on fasting plasma glucose (187). NODAT was diagnosed if FPG was $\geq$7.0 mmol/l or pharmacological treatment was used for treating hyperglycaemia.

Cardiovascular cause of death was defined as death due to myocardial infarction, cerebral haemorrhage, cerebral infarct or cardiac arrest due to unknown cause. This information was obtained directly from case-notes or from death certificates.

2.1.4 IMMUNOSUPPRESSION REGIMEN

Patients randomized to ciclosporin were given Neoral® (Novartis Pharma AG, Basel, Switzerland) 8 mg/kg/day, in two divided doses to maintain trough drug levels of 150-250 ng/ml for the first postoperative month and 100-150 ng/ml thereafter. Individuals randomized to tacrolimus were given Prograf® (Astellas Pharma, Tokyo, Japan) at a dose of 0.2 mg/kg/day, in two divided doses with target trough levels of 5-15 ng/ml for the first month and 5-10 ng/ml thereafter. In addition to the primary agents, both groups also received

methylprednisolone (500 mg) intravenously per-operatively. Both groups received azathioprine (1.5 mg/kg/day) and prednisolone (20mg/day). Prednisolone dose was tapered so that all patients not experiencing an acute rejection episode were steroid-free by three months, whilst individuals experiencing rejection continued taking 5 mg/day for at least one year.

All clinically suspected acute rejection episodes were confirmed by an ultrasound-guided allograft biopsy, with histological classification carried out according to the Banff '97 criteria (188). All patients with histologically confirmed AR episodes were treated initially with intravenous steroid boluses (500 mg of methylprednisolone) on three consecutive days. Steroid-resistant rejection was defined as failure of S. creatinine to fall to the baseline level and histological evidence of ongoing rejection. For patients randomized to ciclosporin, in the presence of steroid resistant rejection, a switch from ciclosporin to tacrolimus was performed, and in the case of further rejection episodes, azathioprine was substituted by MMF. In the presence of persisting rejection, ATG was given. Patients in the tacrolimus arm of the study who experienced steroid resistant rejection were commenced on MMF followed by ATG therapy if required.

2.2 OGTT STUDY COHORT

2.2.1 STUDY DESIGN AND POPULATION

One hundred and twenty-two non-diabetic KTRs with fasting plasma glucose levels between 5.6 mmol/l and 6.9 mmol/l (IFG by ADA criteria) underwent an OGTT as part of a service improvement project in 2005-2006 (181). This was done in order to identify more patients with IFG and IGT and institute lifestyle measures to attenuate CV risk. Following on from the results of this project and in keeping with published international guidelines (109), OGTT was undertaken periodically in high-risk KTRs (IFG by ADA criteria) in order to identify glucose intolerance in a timely manner.

For the current study, 76 KTRs were identified from the original cohort of 122 subjects whose glucose tolerance status could be compared to baseline (2005-2006) (Figure 2.1). All 76 were still under follow-up at the transplant unit of UHW during 2011-2012 (follow-up). Of the original 122 patients, 19 patients had died, 14 had returned to dialysis or had been re-transplanted, eight were lost to follow-up due to transfer to other centres, and five patients did not undergo repeat OGTT due to transportation difficulties. In all, 70/76 patients underwent a repeat OGTT during routine clinic visits and six patients who were on pharmacological treatment for NODAT did not undergo a repeat OGTT. Immunosuppression regimens are explained in detail in sections 2.3.3 and 2.3.4.

Figure 2.1 - Selection of patients for the OGTT cohort study

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┌─────────────────────────────┐
│       Baseline OGTT         │
│         2005-2006           │
│          N=122              │
└─────────────────────────────┘
              │
              │        ┌──────────────────────────────────────┐
              │        │ Died n=19                            │
              │        │                                      │
              ├───────▶│ Return to dialysis or re-transplanted│
              │        │ n=14                                 │
              │        │ Transfer to other centres n=8        │
              │        │ Refused OGTT due to                  │
              │        │ transportation difficulties n=5      │
              │        └──────────────────────────────────────┘
              ▼
┌─────────────────────────────┐
│     Follow-up OGTT study    │
│         2011-2012           │
│           N=76              │
│  70 underwent OGTT, 6 deemed │
│   diabetic as on medication  │
└─────────────────────────────┘
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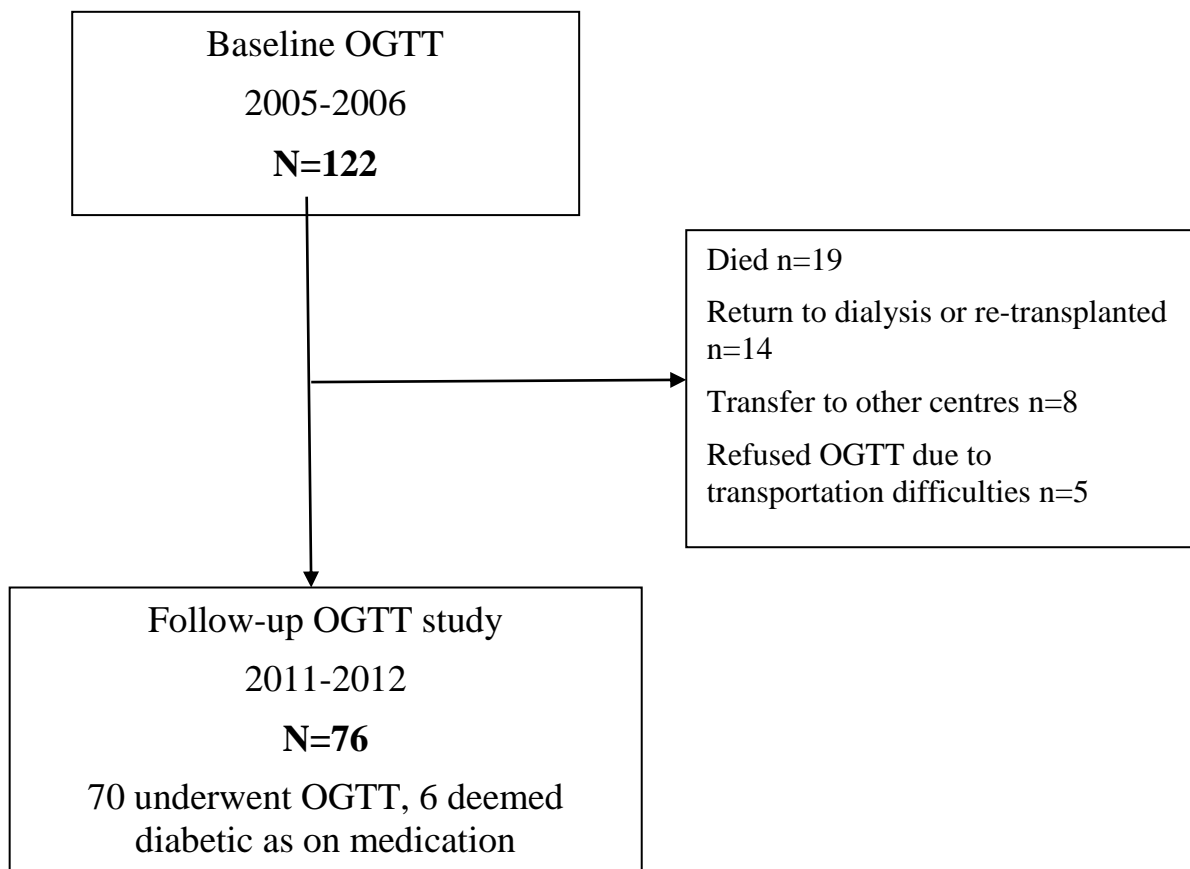
2.2.2 PROCEDURE FOR ORAL GLUCOSE TOLERANCE TEST

OGTTs were undertaken during routine clinic visits. After an overnight 12-hour fast, blood samples were taken for glucose and lipids in addition to routine clinic blood tests. Patients were then administered 75g of glucose (113 ml of Polycal), with post-prandial samples taken two hours after the administration of glucose. Glucose samples were taken in containers with sodium fluoride as the antiglycolytic agent.

2.2.3 DEFINITIONS

Glucose tolerance was determined from the results of the OGTT according to WHO classification (Table 2.1) (187). NODAT was diagnosed if 0-hour glucose was ≥7.0 mmol/l, 2-hour glucose was ≥11.1 mmol/l or anti-diabetic medication was used. MS was determined at baseline based on the International Diabetes Federation (IDF) criteria (Table 2.2) (148). We used waist-hip ratio (WHR) > 0.9 in men and > 0.8 in women as a marker of central obesity.

Table 2.1 – Oral glucose tolerance test classification by the World Health Organisation criteria

	0-hour Glucose mmol/l	2-hour Glucose mmol/l
Normal glucose tolerance (NGT)	<6.1	<7.8
Impaired fasting glucose (IFG)	6.1-6.9	-
Impaired glucose tolerance (IGT)	-	7.8-11
Diabetes mellitus	≥7.0	≥11.1

Table 2.2 – International Diabetes Federation definition of metabolic syndrome

ᵃ Central obesity (Caucasians) (waist circumference)	Male ≥ 94 cm Female ≥ 80 cm
Raised triglycerides	≥ 1.7 mmol/l or specific treatment for this lipid abnormality
Reduced HDL cholesterol	< 1.03 mmol/l in males < 1.29 mmol/l in females or specific treatment for this lipid abnormality
Raised blood pressure	Systolic: ≥ 130 mmHg or Diastolic: ≥ 85 mmHg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	≥ 5.6 mmol/l (100 mg/dl)

ᵃ We used waist-hip ratio >0.9 in males and >0.8 in females as a marker of central obesity

2.2.4 GROUPS AT FOLLOW-UP

At follow-up, patients were divided into two groups based on worsening of OGTT result from baseline – progressors (worsening result) and non-progressors (stable or improved result). Worsening was defined as movement to a higher degree of abnormal OGTT result [NGT → IFG → IGT → IFG & IGT → NODAT]. Patients with NODAT at baseline (n=10) were excluded from this grouping.

2.3 HISTORIC VS. RECENT COHORTS STUDY (INCIDENCE STUDY)

2.3.1 STUDY DESIGN

This was a retrospective observational epidemiologic study of patients who received a kidney transplant in the CTU at UHW. Between January 1997 and December 2001 (old era), 332 patients received a kidney-only transplant, and between January 2004 and December 2009 (new era), 541 received a kidney-only transplant. For the incidence study, exclusion criteria in both eras were: 1. Patients with multi-organ transplants, 2. Death or graft failure within six months after transplantation, 3. Lack of relevant data at 1-year post-transplantation to diagnose NODAT and 4. History of pre-transplant DM [medication use or FPG >7.0 mmol/l and/or random plasma glucose >11.0 mmol/l on two occasions in the year before transplantation]. The R&D Unit of UHW and the South East Wales Research Ethics Committee approved the study.

2.3.2 DEFINITIONS

Using FPG results, fasting glucose tolerance was determined according to WHO classification (187) as follows: NFG = FPG <6.1 mmol/l, IFG = FPG 6.1-6.9 mmol/l and NODAT = FPG ≥7.0 mmol/l or use of anti-diabetic medication. The cumulative incidence of NODAT was calculated up to five years post-transplantation. The date of diagnosis of NODAT was noted in order to calculate NODAT-free survival times.

2.3.3 IMMUNOSUPPRESSION REGIMEN IN THE OLD ERA

Immunosuppression policy was in transition during this era. Table 2.3 shows a summary of the immunosuppression protocol during this period. Decision on the type of CNI used was left to the physician's discretion, except in cases of patients enrolled into randomised trials

which the unit was undertaking during the period. Ninety-one patients received ciclosporin starting at 8 mg/kg/day, in two divided doses to maintain trough levels of 150-250 ng/ml for the first month and 100-150 ng/ml thereafter. One-hundred and thirty three patients received tacrolimus starting at 0.2 mg/kg/day, in two divided doses with target trough levels of 5-15 ng/ml for the first month and 5-10 ng/ml thereafter. Twenty-six patients received sirolimus. In addition to the primary agents, all patients received methylprednisolone (500 mg) i.v. per-operatively followed by prednisolone 20 mg/day orally. Prednisolone dose was tapered according to departmental protocol with an aim to stop by three months post-transplantation in the absence of a contraindication to steroid withdrawal. Prednisolone (5 mg/day) was continued up to a year after a biopsy-proven AR episode, or longer after multiple ARs or ARs requiring ATG for resolution. One hundred and thirty-six patients received azathioprine (starting dose 1.5 mg/kg/day) and 47 patients received MMF starting at 1g twice a day.

All patients with biopsy-proven AR were treated with methylprednisolone 500 mg i.v. on three consecutive days. For patients receiving ciclosporin, in the presence of steroid resistant rejection, a switch from ciclosporin to tacrolimus was performed, and in the case of further rejection episodes, azathioprine was substituted by MMF. In the presence of persisting rejection, ATG was given. Patients receiving tacrolimus who experienced steroid resistant rejection had their therapy optimized and were commenced on MMF if not already on this, followed by ATG therapy if required.

2.3.4 IMMUNOSUPPRESSION REGIMEN IN THE NEW ERA

Induction immunosuppression therapy was used for deceased donor transplants in the form of ATG for DCD transplants or the IL-2 receptor antagonist basiliximab for DBD transplants. Standard maintenance therapy was with tacrolimus, MMF and prednisolone. As a result, 350

patients received tacrolimus, nine received ciclosporin and seven received sirolimus. The starting dose of tacrolimus was 0.2 mg/kg/day at the beginning of the new era period. This was progressively reduced to 0.1 mg/kg/day for transplants in the latter part of the new era. Target trough tacrolimus concentrations were aimed for 10-15 ng/ml for the first three months followed by 5-10 ng/ml thereafter. From January 2008, the target concentration was reduced to 6-9 ng/ml for the first three months and 4-7 ng/ml thereafter for DBD transplants. For DCD recipients, tacrolimus starting dose was reduced to 0.05 mg/kg/day, aiming for a trough tacrolimus concentration of 4-7 ng/ml from the time of transplantation. MMF was initiated at 1g twice a day. Steroid use and treatment of acute rejection were as in the old era.

Table 2.3 – Immunosuppression protocols in the two eras

		1997-2001 Old era	2004-2009 New era
Induction agent		None	Basiliximab for DBD transplants ATG for DCD transplants
Primary agent starting dose		Tacrolimus 0.2 mg/kg/day Ciclosporin 8 mg/kg/day	Tacrolimus 0.2 mg/kg/day From 2006: 0.15 mg/kg/day From 2008: 0.1 mg/kg/day for DBD and 0.05 mg/kg/day for DCD transplants
Target trough blood levels (ng/ml)	**0-3 months**	Tacrolimus 10-15 Ciclosporin 150-250	Tacrolimus 10-15 From 2008: 6-9 for DBD and 4-7 for DCD transplants
	> 3 months	Tacrolimus 5-10 Ciclosporin 100-150	Tacrolimus 5-10 From 2008: 4-7
Secondary agent		Azathioprine 1.5 mg/kg/day MMF 1g twice/day	MMF 1g twice/day
Steroid		20 mg/day, stopped by 3 months	20 mg/day, stopped by 3 months

DCD=donation after circulatory death, DBD=donation after brain-stem death, ATG=anti-thymocyte globulin,

MMF=mycophenolate mofetil

2.4 DATA COLLECTION

Follow-up data for all three studies were obtained from the renal databases Proton and VitalData used in UHW. These databases gather data automatically and prospectively from the haematology and biochemistry laboratory systems in UHW, for all patients with at least one contact with the nephrology and transplant unit. Medication data is entered prospectively manually by the transplant unit staff. Patient death, graft loss and transfer to other centres data is also recorded by the transplant unit staff.

For the Cic vs. Tac follow-up study, the original database of the clinical trial was provided by Mr. Gareth Morris-Stiff. Baseline and follow-up metabolic parameters data were extracted from this database manually for all patients included in the current study. Fasting metabolic parameters including plasma glucose, insulin and triglyceride levels which were measured pre-transplantation, and then at three and twelve months after transplantation as part of the original trial were recorded. Survival data were collected until 31/05/2011, loss of graft or death.

For the OGTT follow-up study, the list of the patients who underwent OGTTs at baseline in 2005-2006 and their results were provided by Dr Adnan Sharif. From this list, patients for the follow-up study were identified using VitaData (Figure 2.1). For both baseline and follow-up, the following parameters were measured and recorded during transplant clinic visits –

- BMI (calculated as kg/m^2)

- WHR (calculated as waist circumference divided by hip circumference, measured according to the WHO guidelines)

- Fasting plasma lipid profile

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- eGFR (calculated using the Modification of Diet in Renal Disease [MDRD] formula)

- Arterial blood pressure (measured using a standard manual mercury sphygmomanometer with the patient seated)

- Anti-hypertensive drug use

- Current immunosuppression regimen and CNI trough blood levels

Data for the incidence study were extracted from the VitalData database. Data were cleaned using MS Excel before analysis using SPSS software. Acute rejection data were extracted manually based on the use of methyprednisolone and on histopathological findings recorded on electronic medical records. Data collected for the incidence study included the following parameters at six and twelve months post-transplantation and annually thereafter –

- Fasting plasma glucose and lipid profile

- BMI

- Immunosuppression and steroid use

- Biopsy-proven acute rejection episodes

- Blood pressure

- CNI trough blood levels

- eGFR according to the MDRD formula

Follow-up was until 23/04/2004 for old era patients, 23/04/2012 for new era patients, and until death, loss of graft or loss to follow-up in both eras.

2.5 EXCLUSION OF PRE-TRANSPLANT DIABETES MELLITUS

Critical to the accuracy of the results of the studies was the correct exclusion of patients with DM pre-transplantation, or before their inclusion in the studies. For the OGTT study, this was done using FPG during a routine clinic visit, followed by an OGTT, which is considered to be the method of choice for diagnosing DM. For the Cic vs. Tac follow-up study and incidence studies, pre-transplant diabetes was diagnosed if any of the following were present –

a. DM mentioned as the cause of ESRF or as co-morbidity

b. Anti-diabetic medication use prior to transplantation

c. FPG >7.0 mmol/l and/or random plasma glucose >11.0 mmol/l on at least two occasions in the year before transplantation. If a non-diabetic plasma glucose value was available only on one occasion, then these patients were deemed non-diabetic.

2.6 STATISTICAL ANALYSIS

Normally distributed continuous data were analyzed using t-tests and ANOVA. Non-normally distributed data were analysed using Wilcoxon signed-rank test or Mann-Whitney U test. Categorical data were analyzed using Chi-squared test or Fisher's exact test as appropriate. Patient and graft survival were analyzed using Kaplan-Meier (K-M) method and Cox's proportional hazards regression model. Risk factors for NODAT and progression of glucose intolerance in the OGTT study were identified using a logistic regression model. Factors with a p-value <0.1 for the odds ratio in univariate analysis were entered into the

multivariate model. PASW18 (SPSS Inc., Chicago, IL) was used for statistical analysis. A p-value of <0.05 was used to determine statistical significance.

2.6.1 ADDITIONAL STATISTICAL ANALYSIS

In the OGTT follow-up study, FPG values at baseline were used to create cut-off points from 5.1 mmol/l through to 6.1 mmol/l at intervals of 0.1. The categorical variables thus obtained were used in the logistic regression analysis.

In the incidence study, NODAT-free survival times were calculated and compared between the old and new eras using the K-M method. Factors affecting the occurrence of NODAT were analysed using logistic regression.

CHAPTER 3: RESULTS FROM THE CIC VS. TAC STUDY

3.0 INTRODUCTION

The aims of this study are described in section 1.12, and the methods in section 2.1.

Briefly, the hypotheses being tested in this study are -

a)   Fasting insulin indices can be used to describe the pathophysiology of NODAT and they can predict the development of NODAT.

b)   Early-diagnosed NODAT exerts a negative impact on patient and graft outcomes.

Therefore, the aims of the study were -

a)   To describe the pathophysiology of NODAT in KTRs using fasting insulin indices.

b)   To determine whether these indices calculated pre-transplantation or early post-transplantation can predict the development of NODAT.

c)   To identify the risk factors associated with the development of early ($<$12 months) NODAT.

d)   To determine the effect of early-onset NODAT on long-term patient survival.

We used the Cic vs. Tac study cohort to investigate the above hypotheses and aims. Patients in this study had fasting metabolic parameters measured which were used to calculate fasting insulin indices, and to diagnose NODAT based on FPG. Also, these patients have now had a follow-up period of >10 years after randomisation, which gives us a good opportunity to study the long-term effects of NODAT diagnosed early after transplantation.

3.1 INCIDENCE OF NODAT, BASELINE AND DEMOGRAPHIC CHARACTERISTICS

From the original Cic vs. Tac trial, 118 patients deemed non-diabetic pre-transplantation were identified for the current follow-up study. Median follow-up time was 11 years (range 10-15 years). The cumulative incidences of NODAT at three months, twelve months and ten years post-transplantation were 25 (21%), 44 (37%) and 48 (42%) respectively. Baseline characteristics of patients who developed NODAT in the first year and those who remained non-diabetic are shown in Table 3.1. Only two patients were non-caucasian. Patients who developed NODAT were significantly older at transplantation (study entry) than those who remained non-diabetic (mean 52±12 vs. 46±14 years, p=0.01). There was no difference in the BMI between the two groups at transplantation (NODAT mean 26.2±4.5 vs. non-NODAT 26.1±4.2, p=0.9). The proportion of men in the NODAT group was numerically higher than in the non-NODAT group (73% vs. 60%, p=0.14).

Table 3.1 – Baseline characteristics of recipients who developed NODAT and those who remained non-diabetic in the first year after transplantation

	NODAT (n=44)	Non-NODAT (n=74)	p
Age at transplant in years (mean ± 1SD)	52±12	46±14	0.01
Gender, males n (%)	32 (73)	44 (60)	0.14
BMI in kg/m² (mean ± 1SD)	26.2±4.5	26.1±4.2	0.9
Fasting plasma glucose (mmol/l, mean ± 1SD)	5.4±0.9	5.3±0.7	0.55
Fasting plasma insulin in mU/l, median (range)	12 (3-41)	14 (3-43)	0.15
Fasting triglycerides in mmol/l, median (range)	1.8 (0.4-5.5)	1.7 (0.5-7.5)	0.85
Fasting high density lipoprotein (mmol/l, mean ± 1SD)	1.02±0.36	1.1±0.32	0.24
Baseline CNI (n) — Ciclosporin	18/44 (41%)	42/74 (57%)	0.09
Baseline CNI (n) — Tacrolimus	26/44 (59%)	32/74 (43%)	

3.2 TYPE OF CNI AND ACUTE REJECTION EPISODES: RELATIONSHIP WITH NODAT

At baseline, of the 118 non-diabetic patients analysed in this study, 58 were randomised to tacrolimus and 60 to ciclosporin. On intention-to-treat analysis, 26 patients randomised to tacrolimus developed NODAT in the first year compared with 18 of the 60 patients randomised to ciclosporin (45% vs. 30%, p=0.09).

As a result of AR episodes and drug intolerance, nine patients were converted from ciclosporin to tacrolimus in the first three months. Five of these nine patients were diagnosed with NODAT by the time of the conversion. Therefore at three months post-transplantation, 67 patients were being treated with tacrolimus and 51 with ciclosporin. Of these, 93 patients still had a normal fasting glucose at three months post-transplantation. On per-protocol analysis of these 93 patients on the basis of the type of CNI at three months, 11/43 patients on tacrolimus and 8/50 patients on ciclosporin developed NODAT by 12 months post-transplantation (26% vs. 16%, p=0.25).

By the end of follow-up, 75 patients had been exposed to tacrolimus. The overall incidence of NODAT in these patients was 48% compared with 28% in those 43 who had never been exposed to tacrolimus (p=0.03). Although the proportion of patients experiencing at least one AR episode in the first year was higher in patients developing NODAT than in non-diabetic patients, this was not statistically different [22 (50%) vs. 27 (37%), p=0.15]. Nine recipients suffered two rejection episodes (6/44 with NODAT vs. 3/74 with no NODAT) and one recipient suffered three rejection episodes (NODAT group). The mean number of AR episodes per patient in the first year was significantly higher amongst NODAT patients (0.7 vs. 0.4, p=0.04). In 20 out of 22 patients with NODAT and AR, the rejection episode occurred before the diagnosis of NODAT.

There was no difference in the proportion of patients receiving prednisolone at three months (NODAT 73% vs. non-NODAT 76%, p=0.7) or one year (NODAT 56% vs. non-NODAT 49%, p=0.5).

Nine patients were treated with anti-diabetic medication during follow-up; all nine patients were on tacrolimus at baseline and at follow-up.

3.3 DIFFERENCES IN INSULIN RESISTANCE AND SECRETION INDICES BETWEEN NODAT AND NON-NODAT PATIENTS

Table 3.2 shows the pre-transplant, three month and twelve month values for insulin indices in patients who developed NODAT and those who remained non-diabetic in the first year after transplantation. Pre-transplant values of HOMAsec, IR-HOMA and McAuley's index were not statistically different between the two groups of patients. However, baseline

HOMAsec appeared to be lower in the group of patients who went on to develop NODAT compared with those who remained non-diabetic (median 131 vs. 166, p=0.18), although this was not statistically different.

At both three and twelve months after transplantation, patients who developed NODAT had a significantly higher IR-HOMA compared with non-diabetic patients (median 6.0 vs. 2.8, p=0.008 and 4.8 vs. 2.6, p=0.01 respectively). At 12 months, HOMAsec was lower in patients who developed NODAT when compared to those who remained non-diabetic (median 88 vs. 125, p=0.05), and this difference just reached statistical significance.

Recipients who suffered AR and developed NODAT had an early increase in insulin resistance: by three months (Table 3.3). In contrast, those recipients who did not suffer AR but still developed NODAT, demonstrated a higher insulin resistance only by 12 months post-transplantation (Table 3.4).

There were no differences in the lipid profile, BMI or eGFR between NODAT and non-diabetic patients at any time point (Table 3.5).

Table 3.2 – Comparison of glucose and insulin parameters at baseline, 3 months and 12 months between recipients who developed NODAT and those who remained non-diabetic in the first year (values expressed as median (range) or mean±1 SD)

	Pre-transplantation			3 months			12 months		
	NODAT n=44	No NODAT n=74	p	NODAT n=44	No NODAT n=74	p	NODAT n=40[a]	No NODAT n=74	p
IR-HOMA	3.0 (0.6-11.0)	3.3 (0.8-10.7)	0.18	6.0 (1.0-40.0)	2.8 (1.1-13.1)	0.008	4.8 (0.6-32.0)	2.6 (0.7-9.5)	0.01
HOMAsec	131 (41-666)	166 (40-916)	0.18	136 (30-2340)	135 (31-896)	0.29	88 (23-500)	125 (36-1953)	0.05
McAuley's Index	5.6 (3.1-11.0)	5.4 (3.2-10.6)	0.50	4.7 (2.4-8.3)	5.8 (2.7-9.3)	0.05	5.8 (3.0-12.0)	6.0 (2.7-9.5)	0.40
FPG mmol/l	5.6±1.1	5.5±1.0	0.70	6.9±1.7	5.6±0.7	<0.001	7.1±1.9	5.5±0.8	<0.001
FPI mU/l	12 (3-41)	14 (3-43)	0.15	17 (3-87)	13 (5-161)	0.01	16 (3-154)	13 (4-122)	0.37

[a] 4 patients being treated with anti-diabetic medication are excluded from this analysis

Table 3.3 – Comparison of insulin indices in the subgroup of patients <u>with</u> acute rejection in the 1st year (N=49), subdivided according to NODAT and No NODAT (37 patients had acute rejection within three months)

Median (range)	Baseline			3 months			12 months		
	NODAT (n=20[a])	No NODAT (n=25)	p	NODAT (n=20)	No NODAT (n=25)	p	NODAT (n=20)	No NODAT (n=25)	p
IR-HOMA	2.1 (0.8-15)	3.3 (1.2-10.1)	0.07	6.5 (1.5-40)	2.6 (1.1-9.6)	0.01	3.5 (0.6-6)	2.2 (0.7-34)	0.67
HOMAsec	96 (43-861)	170 (45-640)	0.07	135 (25-498)	133 (31-489)	0.94	70 (31-225)	124 (43-1953)	0.15
McAuley's index	5.9 (1.7-10.9)	4.5 (3.2-8)	0.06	4.4 (2.3-6.3)	5.9 (4-9.1)	0.02	5.9 (4.6-12)	5.9 (2.6-8.9)	0.5

[a] 4 patients receiving anti-diabetic medication in the 1st year were excluded, but results did not change when such patients were included in the analysis

Table 3.4 – Comparison of insulin indices in the subgroup <u>without</u> acute rejection in the 1st year (N=69), subdivided into NODAT and No NODAT groups

Median (range)	Baseline			3 months			12 months		
	NODAT (n=20)	No NODAT (n=49)	p	NODAT (n=20)	No NODAT (n=49)	p	NODAT (n=20)	No NODAT (n=49)	p
IR-HOMA	3.8 (0.6-15.7)	3.1 (1-10.7)	0.61	4.7 (1-63)	2.5 (1.2-35)	0.42	6.3 (1.6-49)	3.4 (0.7-26)	0.01
HOMAsec	172 (41-665)	168 (47-916)	0.68	112 (15-2340)	130 (33-2296)	0.65	88 (27-831)	128 (36-1553)	0.40
McAuley's index	5.8 (3-9)	5.6 (3.2-9.3)	0.54	5.4 (2.8-8.2)	5.8 (2.4-9.3)	0.62	5.3 (2.5-8)	5.9 (2.6-9.5)	0.18

Table 3.5 – Comparison of metabolic parameters at baseline, 3 months and 12 months between recipients who developed NODAT and those who remained non-diabetic in the first year (values expressed as median (range) or mean±1SD)

	Pre-transplantation			3 months			12 months		
	NODAT n=44	Non-NODAT n=74	p	NODAT n=44	Non-NODAT n=74	p	NODAT n=44	Non-NODAT n=74	P
S. cholesterol mmol/l	5.6±1.5	5.4±1.3	0.58	5.3±1.5	5.7±1.1	0.15	5.6±1.4	5.5±1.2	0.74
S. triglycerides mmol/l	1.8 (0.4-5.5)	1.7 (0.5-7.5)	0.85	1.8 (0.8-5.4)	2.0 (0.8-6.4)	0.56	2.1 (0.7-5.2)	1.5 (0.5-13.2)	0.51
S. low density lipoprotein mmol/l	3.3±1.2	3.4±1.1	0.74	3.2±1.2	3.3±1.0	0.62	3.1±1.0	3.3±1.0	0.52
eGFR ml/min				57±24	55±17	0.54	60±26	59±19	0.90
Body mass index kg/m^2	26.2±4.5	26.1±4.2	0.90	26.3±4.3	26.2±4.3	0.93	27.1±4.7	26.9±4.4	0.88
B. haemoglobin g/dl	9.8±1.8	10.1±1.9	0.46	11.7±1.6	11.7±1.8	0.90	13.4±1.8	13±1.8	0.24

3.4 WITHIN-GROUP DIFFERENCES IN INSULIN RESISTANCE AND SECRETION INDICES

In within-group analyses, amongst NODAT patients, HOMAsec did not change significantly from baseline to three and twelve months (median 131 vs. 136, p=0.37 and 131 vs. 88, p=0.85 respectively). However, IR-HOMA at three months and twelve months was significantly higher compared with baseline (median 6.0 vs.3.0, p=0.001 and 4.8 vs.3.0, p=0.008 respectively). In contrast, amongst the non-diabetic patients, neither HOMAsec nor IR-HOMA changed significantly by three months and twelve months when compared with baseline (median HOMAsec 166, 135, 125 and median IR-HOMA 3.3, 2.8, 2.6 at baseline, 3 months and 12 months respectively; p>0.05 for all comparisons with baseline).

3.4.1 CHANGE IN INSULIN RESISTANCE FROM PRE-TRANSPLANTATION AND CORRELATION WITH NODAT

In an initial analysis, we ranked all patients into two groups based on the median pre-transplant IR-HOMA: low (n=58) and high (n=60). The proportion of patients developing NODAT in the first year was no different in the two groups (low 40% vs. high 35%, p=0.60).

We then calculated the change in IR-HOMA from pre-transplant to three months post-transplant for all patients, and divided the cohort into two groups based on an increase (n=68) or decrease (n=50) in IR-HOMA. The first year incidence of NODAT was significantly higher amongst patients with an increase in IR-HOMA, compared with those with a decrease (46% vs. 20%, p=0.007). Comparisons of other parameters between these two groups are shown in Table 3.6. There was a higher proportion of patients randomised to receive tacrolimus in the group that had an increase in IR-HOMA, however this did not reach statistical significance.

Table 3.6 - Comparison of parameters in patients divided according to the change in IR-HOMA from pre-transplantation to three months post-transplantation

	Increase (n=68)	Decrease (n=50)	p
Age in years (mean ±1SD)	46.9±13	48.2±12	0.63
Pre-transplant BMI (mean ±1SD)	26.3±4	25.6±3	0.35
BMI at 3 months (mean ±1SD)	26.6±4	25.5±3	0.18
Change in BMI from baseline to 3 months (mean±SE[1])	0.27±0.25	-0.9±0.3	0.36
Baseline tacrolimus use, n (%)	39 (58%)	20 (39%)	0.06
Prednisolone use, n (%)	48 (70%)	40 (80%)	0.24

[1] SE – standard error

3.5 CHANGES IN INSULIN PARAMETERS ACCORDING TO BASELINE CNI

In patients treated with tacrolimus, IR-HOMA was significantly higher at three months and twelve months compared with baseline, whereas this was not the case in patients treated with ciclosporin (Table 3.7). HOMAsec and McAuley's index at 3 months and 12 months were not significantly different compared with pre-transplant values in either CNI group. Furthermore, there was no difference in HOMAsec between tacrolimus and ciclosporin treated patients at three months (median 137 vs. 137, p=0.8) or twelve months (median 115 vs. 125, p=0.5). Patients who switched between CNIs were excluded from this analysis. However, repeating the above analyses on an intention-to treat basis according to the type of baseline CNI did not significantly change the results.

There was no difference in the proportion of patients who were on prednisolone therapy at three months (Cic 75% vs. Tac 72%, p=0.80) or twelve months (Cic 48% vs. Tac 48%, p=0.98).

Table 3.7 – Differences in insulin parameters according to the baseline calcineurin inhibitor[a] (3 month and 12 month median values were compared with baseline values for each CNI)

	IR-HOMA			HOMAsec			McAuley's index		
	Baseline	3 months	12 months	Baseline	3 months	12 months	Baseline	3 months	12 months
Ciclosporin **n=43**	3.9	2.9^b	3.5^b	174	137^b	125^b	5.2	4.8^b	5.6^b
Tacrolimus **n=55**	2.6	3.5^c	4.1^c	133	137^b	115^b	5.8	5.5^b	5.9^b

[a] Recipients who had a switch of CNI within 12 months of transplantation are excluded from this analysis;

[b] $p > 0.05$ compared to baseline, [c] $p < 0.05$ compared to baseline

3.6 RISK FACTORS FOR DEVELOPING NODAT

Logistic regression analysis was carried out to determine factors predicting NODAT. In the first model, developing NODAT in the first year (n=44) was the dependent variable. On univariate analysis, age, baseline CNI and exposure to tacrolimus in the first year were significant predictors of NODAT (Table 3.8). All factors whose odds-ratios had a p value of <0.1 were added to the multivariate analysis. On multivariate analysis, age (per year increase, OR 1.039, p=0.01) and being exposed to tacrolimus in the first year (OR 2.624, p=0.02) remained significant.

In the second model, logistic regression analysis was carried out with NODAT developing anytime after three months as the dependent variable (n=23). In univariate analysis, age (per year increase, OR 1.040, p=0.03) was the only significant factor, with 3-month FPG >5.6 mmol/l being borderline significant (OR 2.676, p=0.06). However on multivariate analyses, both age (OR 1.043, p=0.02) and 3-month FPG >5.6mmol/l (OR 2.969, p=0.04) were significant predictors of NODAT after three months.

Table 3.8 – Logistic regression analysis of factors associated with the development of NODAT within the first year after transplantation (univariate analysis)

Factor	Odds ratio	95% CI	p
Age, per year increase	1.037	1.007-1.068	0.01
Gender, male	1.818	0.809-4.086	0.14
BMI, per kg/m²	1.004	0.920-1.095	0.93
Change in BMI from baseline to 12 months	0.974	0.843-1.124	0.71
Baseline CNI tacrolimus	1.896	0.890-4.041	0.09
CNI switch by 3 months, yes	2.244	0.569-8.846	0.24
Exposure to tacrolimus in the 1st year, yes	2.517	1.14-5.557	0.02
Pre-transplant FPG, per mmol/l	1.176	0.716-1.933	0.52
Pre-transplant FPG>5.6 mmol/l	1.451	0.679-3.099	0.33
Pre-transplant fasting P. triglycerides	1.107	0.894-1.371	0.35
Pre-transplant IR-HOMA	1.038	0.930-1.158	0.50
Pre-transplant HOMAsec	1.000	0.997-1.002	0.67
Pre-transplant McAuley's index	1.046	0.846-1.295	0.67
AR episode in the 1st year, yes	1.741	0.816-3.711	0.15
Prednisolone therapy at 12 months, yes	1.234	0.581-2.624	0.58

Table 3.9 - Logistic regression analysis of factors associated with the development of NODAT within the first year after transplantation (multivariate analysis; all factors with p<0.1 in the univariate analysis were included)

Factor	Odds ratio	95% CI	p
Age, per year increase	1.039	1.008-1.070	0.01
[1]Exposure to tacrolimus in the 1st year	2.624	1.162-5.925	0.02
Baseline CNI tacrolimus	2.017	0.922-4.408	0.08

[1] This variable was added in a model separate from "baseline CNI"

Table 3.10 – Logistic regression analysis of factors associated with the development of NODAT after three months of transplantation (univariate analysis)

(only those patients remaining non-diabetic by three months were included, n=93)

Factor	Odds ratio	95% CI	p
Age, per year increase	1.040	1.002-1.078	0.03
Gender, male	1.326	0.497-3.537	0.57
BMI, per kg/m²	1.041	0.936-1.159	0.45
Change in BMI from 3 months to 12 months	1.053	0.809-1.371	0.70
CNI at 3 months tacrolimus	1.744	0.668-4.555	0.25
Exposure to tacrolimus in the 1st year, yes	1.817	0.665-4.967	0.24
FPG at 3 months	1.748	0.863-3.538	0.12
3 month-FPG >5.6 mmol/l	2.676	0.944-7.586	0.06
Fasting P. triglycerides at 3 months	0.819	0.506-1.326	0.41
IR-HOMA at 3 months	1.052	0.947-1.170	0.34
HOMAsec at 3 months	1.001	1.000-1.002	0.12
McAuley's index at 3 months	0.781	0.517-1.180	0.24
AR episode in the 1st year, yes	1.225	0.472-3.182	0.67
Prednisolone therapy at 12 months, yes	1.193	0.462-3.080	0.57

Table 3.11 - Logistic regression analysis of factors associated with the development of NODAT after three months of transplantation (multivariate analysis; all factors with p<0.1 in the univariate analysis were included)

Factor	Odds ratio	95% CI	p
Age, per year increase	1.043	1.005-1.084	0.02
3-month FPG >5.6 mmol/l	2.969	1.009-8.733	0.04

3.7 SURVIVAL ANALYSIS

During follow-up, 35 patients died (30%). Causes of death are shown in Table 3.12. There was no difference in death rates due to cardiovascular when compared to other causes (p=0.3). There was also no difference in the proportion of cardiovascular deaths between the two groups (p=0.4).

Table 3.12 – Causes of death

	NODAT (n=44)	Non-NODAT (n=74)
Total deaths	19 (43%)	16 (22%)
Cardiovascular	3/19 (16%)	6/16 (37%)
Infection	5/19 (26%)	5/16 (31%)
Malignancy	5/19 (26%)	2/16 (13%)
Other	6/19 (32%)	3/16 (19%)

To test whether pre-transplant FPG had an effect on long-term survival, we divided the patients into two groups – those with NFG (FPG <5.6 mmol/l) and those with IFG (FPG 5.6-6.9 mmol/l) pre-transplantation. There was no difference in long-term survival between the two groups (Figure 3.1, log-rank test p=0.48). This result did not change when FPG >6.1 mmol/l was used as the cut-off for defining IFG.

Figure 3.1 -Kaplan-Meier estimates of patient survival in all recipients according to pre-transplant fasting glucose tolerance (NFG - FPG <5.6 mmol/l; IFG - FPG 5.6-6.9mmol/l) (log-rank test p=0.48)
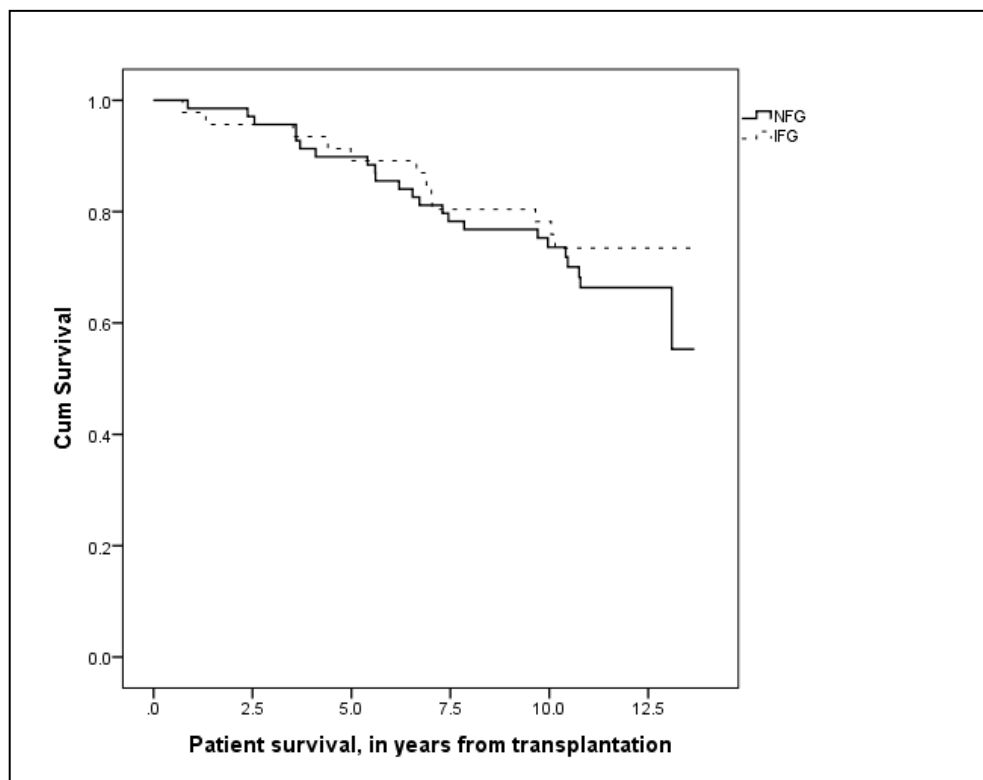
Figure 3.2 shows the Kaplan-Meier estimates for patient survival in patients with and without NODAT in the first year after transplantation. Patients with NODAT had significantly worse survival after a median follow-up of 11 years (log-rank test p=0.008). It is worth noting that the survival curves in Figure 3.2 start to diverge after only about four years post-transplantation.

Figure 3.2 – Kaplan-Meier estimates of patient survival in recipients with and without NODAT in the first year after transplantation (log-rank test p=0.008)
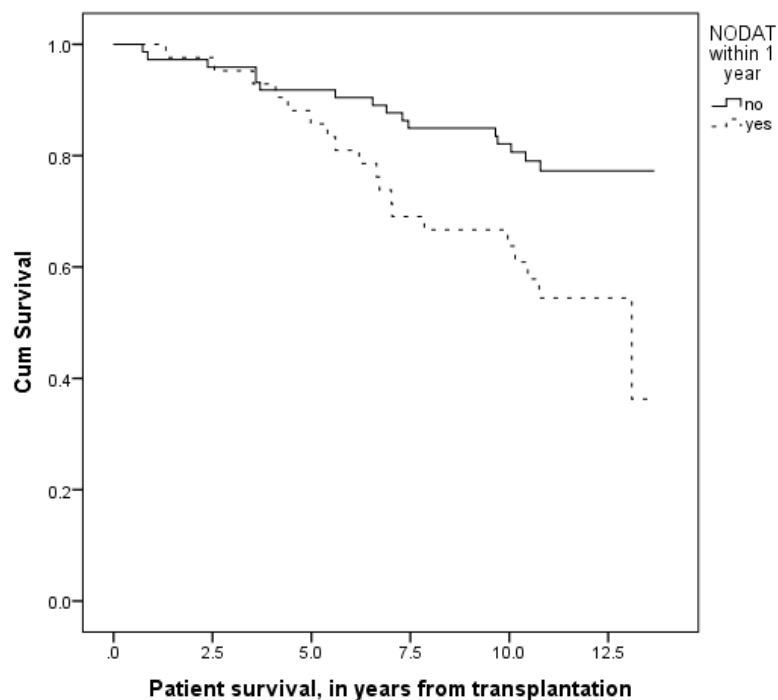
To study the effect of lesser degrees of hyperglycaemia, we divided the patients into three groups based on their fasting glucose tolerance at any time point in the first year after transplantation – NFG, IFG and NODAT according to the WHO classification. As seen in

Figure 3.3, patients with NODAT had inferior survival compared to the other two groups (log-rank test p=0.02). However, there was no difference in survival between the NFG and IFG groups in a separate analysis.

Figure 3.3 - Kaplan-Meier estimates of patient survival in all recipients according to their fasting glucose tolerance at any time point in the first year (log-rank test p=0.02)
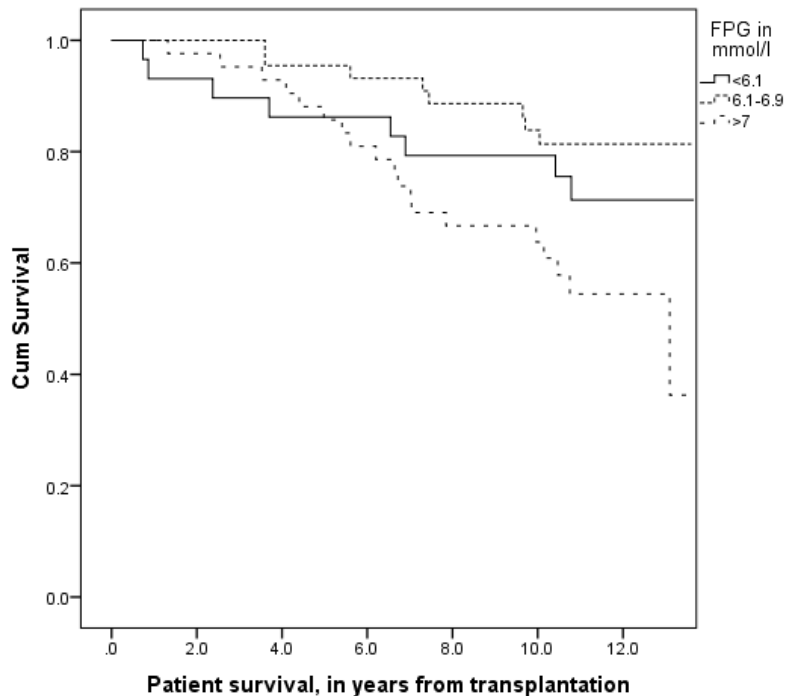
Cox's proportional hazards regression analysis was performed to identify variables correlated with decreased survival (Table 3.13). In univariate analysis, the following variables were associated with decreased survival: higher age at transplant, higher S. creatinine at 12 months, NODAT within 3 months, NODAT within 12 months and prednisolone use at 3 and

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12 months. On multivariate analysis, age, S. creatinine at 12 months and NODAT within 3 months remained as the significant factors. Other variables that did not correlate significantly with patient survival included gender, pre-transplant fasting plasma glucose, fasting plasma glucose at 3 months and 12 months, IR-HOMA at baseline or 12 months, BMI at baseline or 12 months, baseline CNI type, acute rejection episodes, prednisolone use at 3 or 12 months and cumulative methylprednisolone dose.

Table 3.13 – Cox's proportional hazards regression model for all-cause mortality (all factors with a hazard ratio p value of <0.1 in the univariate analysis were entered into the multivariate analysis)

	Univariate			Multivariate		
	Hazard ratio	95% CI	p	Hazard ratio	95% CI	P
Recipient age (per year)	1.07	1.04-1.10	<0.001	1.06	1.02-1.09	0.001
NODAT in 3 months (yes) [1]	2.52	1.27-5.01	0.008	2.26	1.08-4.73	0.03
NODAT in 1 year (yes)	2.24	1.16-4.32	0.016	1.67	0.82-3.38	0.17
S. creatinine at 12 months (per μmol/l)	1.007	1.004-1.009	<0.001	1.007	1.004-1.01	<0.001
Prednisolone use at 3 months (yes) [2]	2.67	0.94-7.56	0.06	2.3	0.68-7.6	0.17
Prednisolone use at 12 months (yes)	2.32	1.1-4.9	0.02	1.12	0.48-2.60	0.78
FPG at 12 months	1.16	0.98-1.38	0.08	1.05	0.86-1.29	0.62
Gender male	1.66	0.77-3.56	0.19	-		
Pre-transplant FPG	0.91	0.59-1.43	0.70	-		
FPG at 3 months	1.11	0.90-1.37	0.31	-		
Pre-transplant BMI	0.96	0.88-1.04	0.29	-		
BMI at 12 months	0.96	0.88-1.03	0.25	-		
AR episodes yes	1.45	0.75-2.81	0.27	-		
Baseline CNI tacrolimus	1.47	0.75-2.9	0.25	-		

[1]Entered into the multivariate model without "NODAT in 1 year"

[2] Entered into the multivariate model without "Prednisolone use at 12 months"

3.8 DISCUSSION

Using simple and validated indices of insulin resistance and secretion, we have demonstrated increasing insulin resistance and a lack of compensatory increase in insulin secretion in patients who developed NODAT following renal transplantation. However, these insulin indices calculated pre-transplantation or early post-transplantation were not able to predict the development of NODAT in multivariate models. Traditional risk factors of age and high plasma glucose, as well as exposure to tacrolimus were associated with NODAT development. The other important finding was that NODAT developing within one year of transplantation was associated with increased all-cause mortality.

Changes in insulin resistance and secretion that occur after renal transplantation have been previously described using complex techniques such as the short insulin tolerance test (85), clamp methods (82) and the OGTT (83, 189). These methods are time consuming, invasive and not practical for large-scale studies or routine clinical use. Using insulin indices such as those calculated from HOMA have an advantage of being simple and easy to use, needing only fasting plasma glucose and insulin tests.

Our findings on changes in insulin parameters detected using HOMA are consistent with other studies that have used gold-standard techniques to measure insulin resistance and secretion (83, 190). Although a decline in insulin secretion has been shown consistently in patients developing NODAT, the evidence for increasing insulin resistance is conflicting. Nam et al., using the insulin sensitivity index derived from the short insulin tolerance test, found that in comparison to pre-transplantation, there was an improvement in insulin sensitivity 9 to 12 months after transplantation in patients who remained normoglycaemic as well as in those who developed NODAT (85). Sato et al., using a modified euglycaemic

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hyperinsulinaemic clamp technique, found no improvement in insulin sensitivity at five weeks after transplantation in patients treated with either ciclosporin or tacrolimus (191). In contrast, Hornum et al. demonstrated deterioration in insulin sensitivity (calculated from an OGTT) by 12 months post-transplantation (192).

In our study, we found that in patients who remained non-diabetic, insulin resistance remained stable post-transplantation, whereas in those who developed NODAT, insulin resistance increased in the first year compared to pre-transplantation (section 3.4). Since insulin resistance and secretion share a hyperbolic relationship with each other (21), lack of a compensatory increase in insulin secretion in patients with NODAT in the presence of increasing insulin resistance suggests pancreatic β-cell deficiency. This is particularly evident by 12 months post-transplantation when HOMAsec is significantly lower in NODAT patients compared with those without NODAT (Table 3.2).

We also found that the increase in insulin resistance that occurs in KTRs who develop NODAT occurs earlier in those who suffer AR, in contrast to a later increase in resistance in those without AR who develop NODAT (Tables 3.3 and 3.4). These results are interesting and support the hypothesis that AR and its treatment with steroids leads to an increase in insulin resistance and thus NODAT in the absence of a corresponding increase in insulin secretion. However, it has to be noted that this is a subgroup analysis and the results have to be interpreted with caution. We could not demonstrate an association between treated AR episodes and the development of NODAT on multivariate analysis (Tables 3.9 and 3.10).

In the general population, insulin resistance and insulin secretion calculated by HOMA have been shown to predict the future development of T2DM (102, 103). However, in one large epidemiological study in Korea, HOMA secretion index did not predict the development of

diabetes (104). In our study, pre-transplant insulin resistance and secretion did not differ between the patients who went on to develop NODAT and those who remained non-diabetic. Also, a high IR-HOMA or low HOMAsec at either baseline or three months post-transplantation was not associated with the development of NODAT on logistic regression analysis. There are a few possible explanations for this negative result. Firstly, this study may have been underpowered to identify the indices as potential risk factors, but the lack of even a trend towards an association, and the fact that median pre-transplant IR-HOMA was in fact numerically lower in the NODAT group, makes this less likely. Secondly, the HOMA equation used to calculate IR-HOMA and HOMAsec contains plasma glucose and insulin. IR-HOMA and insulin level were almost perfectly correlated ($r = 0.98$). Since the level of plasma glucose (as IFG), but not insulin, was a significant factor associated with the development of NODAT, including IR-HOMA in the regression analysis only decreases the effect of glucose. Therefore, insulin resistance and secretion as estimated by HOMA may not predict NODAT in statistical models. Finally, the effects of renal transplantation and immunosuppression on insulin resistance in individual patients are variable. In other words, insulin resistance can change in either direction after transplantation, thus modulating the risk of NODAT. As discussed in section 3.4.1, baseline IR-HOMA did not associate with NODAT, but an increase in IR-HOMA from baseline to post-transplantation did associate with an increased incidence of NODAT. Moreover, the change in GFR post-transplantation may in itself affect glucose and insulin metabolism. Although there is strong evidence for the critical role of the kidney in glucose and insulin metabolism (193-196), the effect of the renal allograft on glucose homeostasis has not been studied.

The incidence of NODAT was 37% in the first year post-transplantation. This incidence is higher compared with that reported by other studies randomizing KTRs to receive either

ciclosporin or tacrolimus (156, 197, 198). Disparities in CNI doses and methodological variations in diagnosing NODAT most likely account for the difference in the incidences of NODAT. We identified NODAT by using fasting plasma glucose measured in the outpatient clinic and by the use of pharmacological therapy. As a result, we labelled as diabetic even those patients who had a FPG $\geq$ 7.0 mmol/l but did not require pharmacological therapy for diabetes, unlike other studies which have used random plasma glucose (115), database recording of DM diagnosis (113, 123) or pharmacological treatment of NODAT (125). Furthermore, the mean age of recipients in our study (48 years) was higher than that reported in other studies (means ranging from 39 to 46.5 years), as was the BMI (mean 26.2 in our study compared to 23.5 to 25.6 in others) (156, 197, 198). Since higher age and BMI are traditional risk factors for T2DM, this may account for the higher incidence of NODAT in our study.

Similar to the finding in the DIRECT study, we noticed that NODAT appeared to be more severe in patients treated with tacrolimus; i.e., all nine patients needing pharmacological therapy for NODAT were on tacrolimus. Although a higher proportion of patients randomized to tacrolimus compared with ciclosporin developed NODAT, this was not found to be statistically different. Since development of NODAT was not an end-point in the original randomised study, our current study may have been underpowered to detect differences in the incidence of NODAT between tacrolimus and ciclosporin groups. Nevertheless, several randomised studies have reported a higher incidence of NODAT in tacrolimus-treated patients compared with cicosporin-treated patients (156, 199-201). However, in our study, having received tacrolimus at any point in the first year was indeed significantly associated with the development of NODAT (increase in odds of 2.6). Clearly,

this analysis was on a per-protocol basis and has less significance than the intention-to-treat analysis.

In tacrolimus-treated patients, IR-HOMA was significantly higher at three months and twelve months compared with baseline (Table 3.7). Despite this increase in IR-HOMA, there was no corresponding increase in HOMAsec. This lack of a compensatory increase in HOMAsec suggests pancreatic β-cell deficiency, presumably as a result of the effect of tacrolimus on β-cells.

In this study, we have also demonstrated a worse long-term patient survival in KTRs who developed NODAT in the first year after transplantation. After 11 years of follow-up, recipients who remained non-diabetic in the first year had a better survival rate than those who developed NODAT within this period. Our use of fasting glucose values to diagnose diabetes is important as we have shown that even NODAT not requiring pharmacological therapy is detrimental to long-term patient survival. A study by Cosio et al. showed that a high plasma glucose level at one year after renal transplantation increased the risk of adverse cardiovascular events, but was not related to long term patient survival (118). In our study, the presence of NODAT at three months or twelve months was significantly associated with mortality in univariate analysis, and NODAT at three months on multivariate Cox regression analysis. We were not able to demonstrate an association between NODAT and death due to cardiovascular causes; this may be due to a lack of statistical power as this was not a primary aim of the original trial.

The mortality associated with early NODAT could be speculated to be a reflection of the severity or strength of immunosuppression, causing adverse outcomes due to infection and malignancy in addition to CV causes. However, in the multivariate Cox analysis, we did not

see any association between mortality and acute rejection, prednisolone use at one year or cumulative dose of methylprednisolone (surrogate markers for the strength of immunosuppression).

Our finding of a lack of association between NODAT and cardiovascular mortality is in contrast to that reported by Hjelmesaeth et al. (117). In their study, cardiovascular mortality in KTRs with NODAT (20%) was higher than that in non-diabetic recipients (8%, p=0.058). Although not statistically significant, there was a clear trend towards higher cardiovascular mortality in KTRs with NODAT. Since they used OGTT in addition to fasting glucose to diagnose diabetes, compared to only fasting glucose in our study, the difference in cardiovascular mortality in the two studies may be a reflection of the adverse effect of post-prandial hyperglycaemia. However, the factors we found significantly associated with death (increased age, high S. creatinine level and diabetes) are also the traditional CV risk factors.

The strengths of our study include the long duration of follow-up with no losses to follow-up, use of fasting rather than random metabolic parameters, and the use of WHO criteria for the diagnosis of diabetes. Our study has certain limitations. Firstly, OGTT was not performed prior to transplantation. This may have led to underestimation of the burden of T2DM at baseline and over-estimation of early NODAT due to unravelling of previously undiagnosed T2DM. A similar issue may have arisen post-transplantation by using fasting plasma glucose rather than an OGTT. Secondly, the relationship between GFR, glucose and insulin levels is complex (202). HOMA equations contain fasting plasma glucose and insulin measurements. Therefore, using HOMA to estimate insulin resistance and secretion in patients with renal disease may be simplistic, but is the most practical method in order to avoid more invasive tests. However, these indices have been validated in dialysis patients and KTRs. Homeostatic

techniques such as HOMA may not accurately reflect the prevailing levels of insulin resistance and secretion. We speculate that dynamic methods such as the OGTT-derived Insulin Sensitivity Index (203) may yield more accurate measurements of insulin sensitivity and thus give rise to differing results. Finally, it is not clear whether the effect of NODAT on increased mortality is a glucose-mediated effect or not. Since we did not have data on HbA1c, we could not study the effect of the level of plasma glucose control on mortality. Similarly, since we did not have data on the cumulative exposure to steroids or CNI, it is not possible to fully study the effect of immunosuppression on mortality. We have partly overcome this limitation in the incidence study (Chapter 5).

In conclusion, using validated insulin indices, we have demonstrated a lack of an appropriate insulin secretory response in the face of increasing insulin resistance in patients developing NODAT in the first year after renal transplantation. However, these indices measured pre-transplantation or at three months after transplantation were not able to predict the development of NODAT. On the other hand, the traditional risk factors for T2DM - higher age and higher plasma glucose levels - were significant predictors of NODAT, along with exposure to tacrolimus. Development of early NODAT was associated with decreased patient survival.

Therefore, based on the results from the Cic vs. Tac cohort study, it appears that tacrolimus plays an important role in the occurrence of NODAT early after renal transplantation, and that older patients are more susceptible to this effect. Two important questions that arise are: 1. Whether this effect holds good late after transplantation and 2. Whether reducing the exposure to tacrolimus by lowering the dosage and target blood levels reduces the risk of NODAT. We address these questions in Chapters 4 and 5.

Another key question is whether the effect of NODAT on mortality can be ameliorated by glucose-lowering therapies. Randomised controlled trials are needed to answer this question.

CHAPTER 4: RESULTS FROM THE OGTT FOLLOW-UP STUDY

4.0 INTRODUCTION

The aims of this study are described in section 1.12 and methods in section 2.2

Briefly, the hypothesis being tested in this study is that late after transplantation, the traditional risk factors for T2DM are more significant than transplant-specific factors in causing NODAT and progressive glucose intolerance.

The aims of this study were -

a) Using OGTTs, to investigate the progression of glucose tolerance abnormalities developing late after transplantation.

b) To identify the risk factors associated with the development of late (>12 months) NODAT.

c) To examine the association between the metabolic syndrome identified late after transplantation and glucose intolerance.

We used the OGTT follow-up study cohort to investigate this hypothesis. As described in section 2.2, this cohort of patients had undergone an OGTT in 2005-2006. A proportion of these patients underwent a follow-up OGTT in 2011-2012 to identify progression of glucose intolerance. BMI, WHR, blood pressure and a fasting blood lipid profile were also measured. Patients with MS were identified using the IDF definition (Table 4.1). The process of selecting patients for this study is shown in Figure 2.1. OGTT results from the two time-points were compared and the risk factors for the progression of glucose intolerance and NODAT determined.

Table 4.1 – The International Diabetes Federation definition of metabolic syndrome (presence of central obesity plus any two of the other four factors)

ª Central obesity (Caucasians) (waist circumference)	Male ≥ 94 cm Female ≥ 80 cm
Raised triglycerides	≥ 1.7 mmol/l or specific treatment for this lipid abnormality
Reduced HDL cholesterol	< 1.03 mmol/l in males < 1.29 mmol/l in females or specific treatment for this lipid abnormality
Raised blood pressure	Systolic: ≥ 130 mmHg or Diastolic: ≥ 85 mmHg or treatment of previously diagnosed hypertension
Raised fasting plasma glucose	≥ 5.6 mmol/l or a previous diagnosis of DM

ª We used waist-hip ratio >0.9 in males and >0.8 in females as the marker of central obesity

4.1 GENERAL RESULTS

Seventy-six of the original 122 non-diabetic KTRs who underwent an OGTT at baseline were studied again at follow-up. Of the 76 patients, 70 underwent repeat OGTT and six patients who were already on treatment for diabetes did not undergo repeat OGTT, but were considered to have NODAT at follow-up. At baseline, median time from transplantation was five years (range 1.2 − 19). Median time between baseline and follow-up OGTTs was six years (range 5.3-6.5 years).

The proportion of patients receiving prednisolone fell from 28% at baseline to 22% at follow-up. At baseline, 45 patients were on tacrolimus (59%) and 22 on ciclosporin, compared with 43 on tacrolimus (57%) and 21 on ciclosporin at follow-up. Mean tacrolimus trough blood levels decreased significantly from baseline to follow-up (10 ± 1.4 ng/ml vs. 7.2 ± 1.8, $p<0.001$), as did mean ciclosporin levels (141 ± 34 ng/ml vs. 98 ± 35, $p<0.001$). None of the patients had Hepatitis C and 97% were caucasians.

4.2 CHANGES IN GLUCOSE TOLERANCE AND CARDIO-METABOLIC PROFILE FROM BASELINE TO FOLLOW-UP

Overall, there were significant increases in both 0-hour and 2-hour plasma glucose levels from baseline to follow-up of the whole cohort (mean 5.7 ± 0.7 to 5.9 ± 0.9 mmol/l, $p=0.03$ and mean 6.7 ± 1.9 to 7.5 ± 2.8 mmol/l, $p=0.03$ respectively).

OGTT results from baseline and follow-up are shown in Figure 4.1. The proportion of patients with an abnormal OGTT result increased from 42% at baseline to 61% at follow-up ($p=0.007$), whilst the prevalence of NODAT increased from 13% at baseline to 24% at follow-up ($p=0.001$). Of the 44 patients with normal glucose tolerance at baseline, 21 (48%) developed an abnormal OGTT result at follow-up (7 IFG, 7 IGT, 3 IFG & IGT and 4 NODAT). Seven of the ten patients with NODAT at baseline continued to have NODAT at follow-up while two reverted to NGT and one to IGT. Six patients were on pharmacological treatment for diabetes at follow-up: Four with NODAT from baseline, and two who developed NODAT during follow-up.

There were increases in BMI and WHR of the entire cohort from baseline to follow-up (BMI mean 28.0 ± 5.6 to 28.9 ± 6.1, p=0.001 and WHR mean 0.95 ± 0.08 to 0.99 ± 0.10, p=0.009). Systolic and diastolic BP were both significantly lower at follow-up compared with baseline (mean 137 ± 16 vs. 127 ± 16, p<0.001 and mean 78 ± 9 vs. 73 ± 11, p=0.003), despite no change in the mean number of antihypertensive medications used (2.0 vs. 2.1, p=0.22). No significant change was seen in S. total cholesterol (baseline mean 4.6 ± 0.9 mmol/l vs. follow-up mean 4.7 ± 0.9 mmol/l, p=0.48) and S. triglyceride level (baseline mean 1.7 ± 0.9 mmol/l vs. follow-up mean 1.6 ± 0.8 mmol/l, p=0.58).

Figure 4.1 - Results of baseline and follow-up oral glucose tolerance tests, classified according to the WHO definition
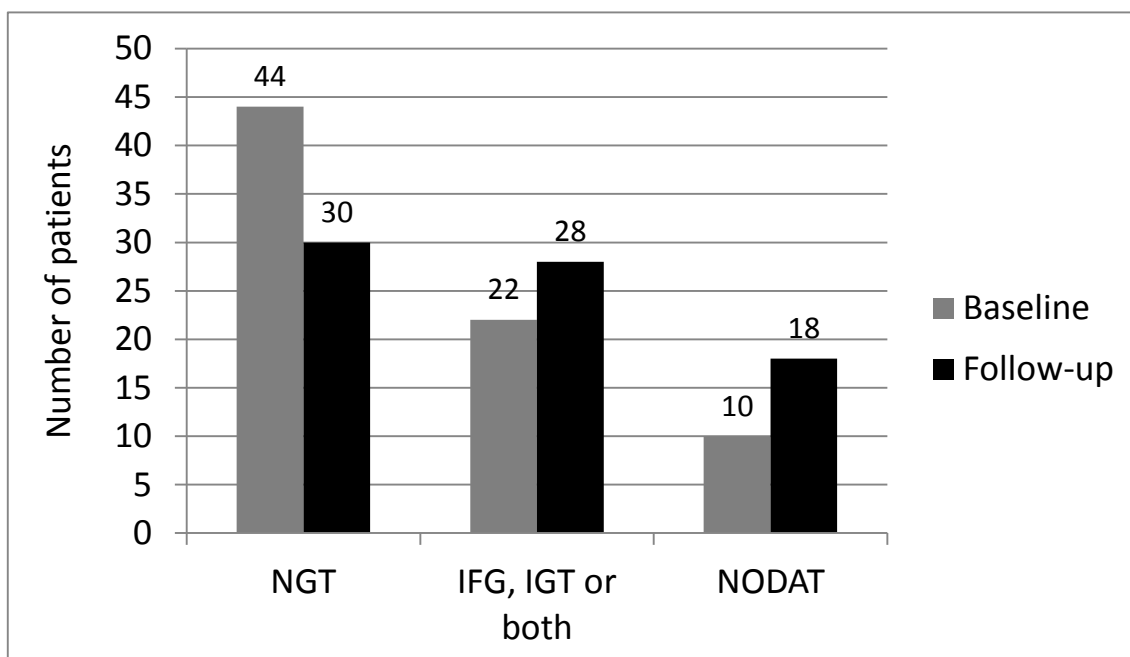
4.3 DIFFERENCES IN CHARACTERISTICS BETWEEN PROGRESSORS AND NON-PROGRESSORS

As mentioned in section 2.2.4, patients at follow-up were divided into two groups based on OGTT results from baseline and follow-up – progressors (worsening result) and non-progressors (stable or improved result). Worsening was defined as a higher degree of abnormal OGTT at follow-up [NGT → IFG → IGT → IFG & IGT → NODAT]. Patients with NODAT at baseline (n=10) were excluded from this grouping.

Table 4.2 shows baseline characteristics of the 28 patients who progressed to a higher degree of glucose intolerance and 38 patients who did not progress. There was no difference in age (mean 58 vs. 56 years, p=0.5), BMI (mean 29.3 vs. 27.1, p=0.15) or WHR (mean 0.96 vs. 0.94, p=0.3) between progressors and non-progressors respectively. Prevalence of steroid use (progressors 21% vs. non-progressors 29% at baseline and follow-up, p=0.49) and tacrolimus use (baseline 54% vs. 58%, p=0.8 and follow-up 54% vs. 53%, p=0.9) was also similar. Baseline MS was more prevalent amongst progressors than amongst non-progressors [19/28 (68%) vs. 14/38 (37%), p=0.01].

Four of the 28 patients who progressed (14%) had a family history of T2DM compared with seven of the 38 non-progressors (18%, p=0.90). Family history was not known for 27 of the 66 patients (27%).

Table 4.2 – Differences in baseline characteristics and medication use between progressors and non-progressors (excluding ten patients with NODAT at baseline) (continuous variables expressed in mean±1SD)

		Progressors n=28	Non-progressors n=38	p
0-hour glucose in mmol/l		5.8±0.5	5.5±0.5	0.05
2-hour glucose in mmol/l		6.5±1.2	6.7±1.9	0.60
Body mass index in kg/m²		29.3±6.8	27.1±4.3	0.15
Waist hip ratio		0.96±0.08	0.94±0.09	0.39
S. cholesterol in mmol/l		4.5±0.8	4.5±0.9	0.90
S. triglycerides in mmol/l		1.8±0.9	1.5±1.08	0.22
eGFR in ml/min		46±15	43±14	0.30
Systolic BP in mmHg		136±14	138±18	0.60
Diastolic BP in mmHg		77±8	77±10	0.90
Prevalence of baseline MS, n		19 (68%)	14 (37%)	0.01
Change in BMI from baseline to follow-up		1.14±2.8	1.01±1.9	0.80
Change in WHR from baseline to follow-up		0.07±0.1	0.05±0.07	0.90
Persisting steroid therapy		6 (21%)	11 (29%)	0.54
Statin use at follow-up, n		19 (68%)	19 (50%)	0.15
Baseline ACEi/ARB use, n		11 (39%)	14 (37%)	0.80
Baseline β-blocker use, n		18 (64%)	23 (61%)	0.75
Current smoking at baseline, n		3 (12%)	3 (9%)	0.75
Baseline trough level in ng/ml	Tacrolimus	10.5±1.5	9.9±1.3	0.24
	Ciclosporin	151±46	133±23	0.33
Follow-up trough level in ng/ml	Tacrolimus	6.5±1.3	6.6±1.3	0.85
	Ciclosporin	122±35	100±18	0.11

4.4 ASSOCIATION BETWEEN METABOLIC SYNDROME AND GLUCOSE TOLERANCE

ABNORMALITIES

At baseline, 41 patients met the IDF criteria for MS. Table 4.3 shows details of these 41 patients in terms of meeting the diagnostic criteria. Ten patients were diabetic on the baseline OGTT, eight with MS and two without MS (p=0.07). When the 66 non-diabetic patients were analysed, 16/33 (49%) with MS and 6/33 (18%) without MS had an abnormal baseline OGTT result.

Of the 33 non-diabetic patients with MS, 19 (57%) progressed to a higher degree of glucose intolerance, as compared to 9 of the 33 (27%) without MS who progressed (p=0.01). Also, the proportion of patients with baseline MS who developed NODAT at follow-up was numerically higher than those without baseline MS (8/33 (24%), vs. 3/33 (9%), p=0.09). Ciclosporin use at baseline was not higher amongst patients with MS compared to those without MS (30% vs. 36%, p=0.57). Within the MS sub-group, those patients with progression of glucose intolerance were older, had a greater increase in BMI and a higher proportion were on tacrolimus; however, none of these differences were significant statistically compared to the non-progressors who had MS at baseline (Table 4.4).

Table 4.3 – Diagnosis of MS at baseline (all patients met the criteria for central obesity, men WHR > 0.9, women WHR > 0.8)

Factor	Number (%) of patients meeting the criteria (N=41)
FPG >5.6 mmol/l or a diagnosis of DM	31 (76%)
Systolic BP ≥ 130 mmHg	28 (68%)
Diastolic BP ≥ 85 mmHg	10 (41%)
Treatment of previously diagnosed hypertension	40 (98%)
Fasting S. triglycerides ≥ 1.7 mmol/l	21 (51%)
Fasting HDL cholesterol < 1.03 mmol/l in males and < 1.29 mmol/l in females	27 (66%)

Table 4.4 – Comparison of characteristics between progressors and non-progressors within the sub-group who had metabolic syndrome at baseline (N=33, data expressed as mean ±1SD)

Baseline parameters	Progressors n=19	Non-progressors n=14	p
Age at transplant, years	49±11	45±14	0.36
BMI, kg/m²	29±6.2	28±3.9	0.66
WHR	0.96±0.07	98±0.07	0.35
S. total cholesterol, mmol/l	4.6±0.6	4.8±0.6	0.37
S. trigycerides, mmol/l	1.8±0.9	1.9±1.6	0.87
Systolic BP, mmHg	139±14	140±15	0.78
Diastolic BP, mmHg	79±7	79±10	0.80
Change in BMI (baseline to follow-up)	1.8±0.2	0.8±0.2	0.65
Change in WHR (baseline to follow-up)	0.04±0.1	0.02±0.09	0.60
0-hr glucose, mmol/l	5.9±0.5	5.8±0.5	0.8
2-hr glucose, mmol/l	6.7±1.2	7.6±1.9	0.10
Tacrolimus use, n	12 (63%)	6 (43%)	0.34
Prednisolone use, n	4 (21%)	6 (42%)	0.25
History of acute rejection, n	6 (33%)	6 (43%)	0.58

4.5 FACTORS ASSOCIATED WITH THE PROGRESSION OF GLUCOSE INTOLERANCE

Table 4.5 shows the results of univariate logistic regression analysis of factors associated with the progression of glucose intolerance. Again, the 66 patients who were non-diabetic at baseline were included in this analysis. In univariate analysis, baseline FPG as a continuous variable, baseline FPG >5.6 mmol/l as a categorical variable and presence of MS as a categorical variable were significant factors with p values for odds ratios <0.1. These factors were then tested in a multivariate model. Since there were significant correlations between each of these three factors, they were entered into the multivariate models separate from each other.

In multivariate logistic regression analysis adjusted for age and gender, MS as a categorical variable was significantly associated with the progression of glucose intolerance (Table 4.6). Adjusted for age and gender, FPG >5.6 mmol/l was also a significant factor whereas FPG as a continuous variable was not.

Table 4.5 – Univariate logistic regression analysis of factors associated with the progression of glucose intolerance (excluding ten patients who were diabetic based on the baseline OGTT)

	Odds ratio	95% CI	p
Age, in years	1.015	0.973-1.059	0.49
Gender, female	1.250	0.393-3.978	0.70
Baseline BMI, per unit	1.077	0.977-1.187	0.13
Baseline WHR, per unit	1.003	0.995-1.009	0.25
Baseline weight, per kg	1.102	0.985-1.040	0.39
Time since transplantation, in years	0.973	0.643- 1.973	0.89
Baseline 0-hr glucose, per mmol/l	2.501	0.977-6.401	0.056
Baseline 2-hr glucose, per mmol/l	0.927	0.687-1.251	0.62
Baseline abnormal OGTT result, yes	0.511	0.175-1,497	0.22
Baseline 0-hr glucose >5.6 mmol/l, yes	4.286	1.497-12.97	0.007
Baseline S. triglycerides, per mmol/l	1.339	0.786-2.281	0.28
Baseline systolic BP, per mmHg	0.994	0.964-1.024	0.67
Baseline diastolic BP, per mmHg	1.000	0.948-1.055	0.99
Baseline MS, yes	3.619	1.290-10.150	0.01
Baseline CNI, tac	0.680	0.040-11.700	0.79
Persisting steroid therapy, no	1.494	0.476-4.68	0.49
History of acute rejection, yes	0.688	0.246-1.920	0.47
Family history of T2DM, yes	0.750	0.187-3.011	0.68

Table 4.6 – Multivariate analysis of factors associated with the progression of glucose intolerance

	Odds ratio	95% CI	p
Age, in years	1.008	0.964-1.055	0.72
Gender, female	1.218	0.359-4.131	0.75
[1]Baseline MS, yes	3.514	1.241-9.946	0.01
[2]Baseline FPG >5.6 mmol/l, yes	4.830	1.575-14.81	0.006
[3]Baseline 0-hr glucose, per mmol/l	2.720	1.001-7.404	0.05

[1], [2] and [3] added to the model independent of each other due to significant correlation

4.6 CHARACTERISTICS OF PATIENTS WHO PROGRESSED TO NODAT DURING FOLLOW-UP

In the previous section, we have been discussing patients who moved between any glycaemic categories. In this section, we will discuss those patients who progressed to an actual diagnosis of NODAT during follow-up.

Of the 66 non-diabetic patients at baseline, 11 (17%) developed NODAT at follow-up. There was no difference in age, gender, baseline BMI and WHR, and tacrolimus or steroid use between patients who progressed to NODAT compared with those who did not (Table 4.7). However, baseline 0-hour mean plasma glucose was significantly higher amongst the patients who went on to develop NODAT compared with those who remained non-diabetic (mean 6.1

vs. 5.5, p=0.003). Seven of the 11 patients who developed NODAT at follow-up had an abnormal OGTT result at baseline and four had a normal OGTT (p=0.03). Again, MS at baseline was more prevalent amongst those who went on to develop NODAT compared with those who did not develop NODAT [8/11 (73%) vs. 25/55 (46 %), p=0.09], but this was not statistically different. Of the 11 patients who developed NODAT, four had a family history of T2DM, four did not, and the family history was unknown for three patients (p=0.16).

Table 4.7 - Differences in baseline characteristics between those who did and those who did not develop NODAT during follow-up (excluding ten patients with NODAT at baseline)

		NODAT during follow-up n=11	No NODAT n=55	p
0-hour glucose in mmol/l		6.1±0.6	5.5±0.5	0.003
2-hour glucose in mmol/l		7.3±1.2	6.5±1.7	0.12
Baseline OGTT	Normal	4 (36%)	40 (73%)	0.03
	Abnormal	7 (64%)	15 (27%)	
Tacrolimus treatment		73%	53%	0.32
Age at baseline, years		48.5±9	51.2±12	0.48
Males, n		7 (64%)	44 (80%)	0.35
Body mass index in kg/m²		30.6±8.6	27.6±4.9	0.33
Waist hip ratio		0.96±0.10	0.95±0.08	0.65
S. cholesterol in mmol/l		4.5±0.75	4.5±0.9	0.78
S. triglycerides in mmol/l		2.1±1.19	1.5±0.95	0.07
eGFR in ml/min		41±12	45±15	0.38
Systolic BP in mmHg		139±15	137±16	0.77
Diastolic BP in mmHg		78±7	77±9	0.88
Prevalence of MS, n		8 (73%)	25 (46%)	0.09
Change in BMI from baseline to follow-up		2.0±2.9	0.9±2.2	0.16
Change in WHR from baseline to follow-up		0.04±0.09	0.05±0.08	0.91

All continuous data in mean±1SD

4.7 FACTORS INDEPENDENTLY ASSOCIATED WITH THE DEVELOPMENT OF NODAT IN LOGISTIC REGRESSION MODELLING

On univariate logistic regression analysis, baseline 0-hr glucose level and an abnormal OGTT result as a categorical variable were the two factors significantly associated with NODAT (Table 4.8)

On multivariate analysis, a high baseline 0-hour glucose level was still significantly associated with the development of NODAT, adjusted for age and gender (OR 10.54, 95% CI 2.27–49.01, p=0.003). Abnormal baseline OGTT also remained significant in the multivariate analysis (Table 4.9).

Table 4.8 – Univariate logistic regression analysis of factors associated with the development of NODAT during follow-up (excluding ten patients who were diabetic at baseline)

	Odds ratio	95% CI	p
Age, in years	0.981	0.928-1.036	0.48
Gender, female	2.286	0.567-9.222	0.24
Baseline BMI, per unit	1.087	0.969-1.219	0.15
Baseline WHR, per unit	1.002	0.995-1.009	0.52
Baseline weight, per kg	1.126	0.992-1.061	0.14
Time since transplantation, in years	0.973	0.823- 1.151	0.74
Baseline 0-hr glucose, per mmol/l	6.180	1.621-23.56	0.008
Baseline 2-hr glucose, per mmol/l	1.344	0.917-1.971	0.12
Baseline abnormal OGTT result, yes	4.660	1.193-18.26	0.02
Baseline 0-hr glucose >5.6 mmol/l, yes	2.974	0.713-12.41	0.13
Baseline S. triglycerides, per mmol/l	1.600	0.908-2.810	0.10
Baseline systolic BP, per mmHg	1.008	0.964-1.024	0.69
Baseline diastolic BP, per mmHg	1.006	0.937-1.080	0.87
Baseline MS, yes	3.200	0.766-13.36	0.11
Baseline CNI, tac	2.391	0.573-9.976	0.23
Persisting steroid therapy, no	0.911	0.212-3.917	0.90
History of acute rejection, yes	0.545	0.130-2.288	0.40
Family history of T2DM, yes	2.857	0.499-16.36	0.23

Table 4.9 – Multivariate logistic regression analysis of factors associated with the development of NODAT during follow-up

	Odds ratio	95% CI	p
Age, in years	0.956	0.889-1.029	0.23
Gender, female	0.173	0.029-1.046	0.05
[1]Baseline 0-hr glucose, per mmol/l	10.54	2.270-49.01	0.003
[2]Baseline abnormal OGTT result, yes	5.159	1.258-21.16	0.02

[1] and [2] added to the model independent of each other due to significant correlation

4.8 DISCUSSION

The principal finding of this study is that there is significant progression of glucose intolerance in kidney transplant recipients even late after transplantation. In multivariate analysis, this progression was associated with a cluster of cardio-metabolic risk factors in the form of MS and FPG >5.6 mmol/l. The deterioration in glucose tolerance was seen in conjunction with increases in WHR and BMI, and a concomitant decrease in steroid use and CNI blood levels. Abnormal baseline OGTT predicted future NODAT.

The overall prevalence of an abnormal OGTT result increased from 42% at baseline to 61% at follow-up (Figure 4.1). This finding is in contrast to that reported by Hagen et al., who found that the proportion of patients with an abnormal OGTT result decreased from 54% at 10 weeks to 35% at six years after transplantation (204). In their study, they performed paired OGTTs over a period of six years in 63 KTRs, with the baseline test at ten weeks post-transplantation, with all patients being treated with ciclosporin. Since the baseline OGTT was very early post-transplant (ten weeks), ciclosporin trough blood levels were quite high (median 255 μg/ml). This may explain the higher incidence of baseline abnormal OGTT results in the Hagen study. As in our study, they also reported an increase in BMI during follow-up, but the median BMI at follow-up (24.3 kg/m²) in their study was lower than the median follow-up BMI in our cohort (28.3 kg/m²).

In KTRs, an abnormal OGTT on day five after transplant has been shown to predict the development of NODAT over a four year period (205). No similar studies exist for the late transplantation period when steroid and CNI use is lower. In our study, an abnormal OGTT result late after transplantation was associated with the development of future NODAT.

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Although in multivariate analysis the confidence intervals were wide (1.2-21.1), this was still statistically significant. Nevertheless, our findings are similar to those of large studies in the general population which have established that IGT and IFG are risk factors for developing T2DM in the future (IGT conferring more risk than IFG) (184, 185).

In this study, as there was no difference in the pattern of CNI use and trough levels between progressors and non-progressors, it was not possible to demonstrate any diabetogenic effect of CNIs in the late post-transplant period. Steroid use was also similar between the two groups. There were increases in BMI and WHR of the whole cohort over time. This might provide indirect evidence of increasing insulin resistance since BMI and WHR correlate with insulin resistance in the general population and in KTRs (100, 206, 207).

The increasing prevalence of NODAT late after transplantation has been noted by a few studies. In a study with a mean follow-up of 3.5 years, Porrini et al. found that the prevalence of NODAT (based on FPG >7 mmol/l or medication use) increased from 8% at baseline to 40% at follow-up (153). About 23% of patients were treated with tacrolimus and the mean BMI of patients was approximately 29 kg/m². In the study by Hagen et al. mentioned above, the prevalence of NODAT (by OGTT) rose from 19% at baseline to 22% at follow-up (204). As noted above, in their study, none were on tacrolimus and the median BMI was only 24.3 kg/m². In our study, the rate of tacrolimus use was 59% at baseline and 57% at follow-up. The mean BMI of 28 kg/m² in our study was also comparable to that in the study by Porrini et al., and we saw an increase in the prevalence of NODAT from 13% to 24%.

As introduced in section 1.8.4.2, MS is a cluster of cardio-metabolic risk factors which increases the risk for developing DM and adverse cardiovascular events. It was first described as a concept in 1988 by Reaven (208). Since then, despite a large amount of research into its

pathophysiology, epidemiology and prognostic importance, controversy still exists related to MS. More recently, some experts have even doubted the existence of MS as a syndrome and called into question its clinical value over and above its individual components (209, 210). Others have defended its value by stating that the recognition of MS helps to identify people who have a risk of developing DM and cardiovascular events (148, 211). This was shown in a meta-analysis that included 172,573 individuals from the general population (212). In this meta-analysis, MS conferred a relative risk of CV events and death of 1.78 (95% CI 1.5-2.0). The association remained after adjusting for traditional CV risk factors (RR 1.54, 95% CI 1.32-1.79). Significantly, the higher risk was seen even in patients without prevalent CV disease. The latter finding is very important in the context of kidney transplantation as we know that NODAT and cardiovascular complications are common problems.

Porrini et al. studied a cohort of 230 KTRs and found a prevalence of MS of 22.6% at baseline (1-year post-transplantation, using BMI as marker of central obesity), which rose to 37.7% over a period of 3-4 years (153). They showed that patients with MS at baseline were more likely to develop NODAT compared with those without MS (log-rank 23.77, p<0.001). MS also increased the risk of overall graft loss (HR 2.8-4.5 in various multivariate models) and death (log rank test, p=0.02). In a slightly different study, Bayer et al. reported a prevalence of MS of 57% pre-transplantation (152). Patients with MS were more likely to develop NODAT compared with recipients without MS (34% vs. 27%, p=0.057). In contrast to these two studies, we analysed patients with IFG by ADA criteria late after transplantation (median six years from transplant at baseline). The prevalence of MS in this selected group of patients was 46%. Furthermore, a large proportion of patients with MS (57%) progressed to higher degrees of glucose intolerance with 24% developing NODAT during follow-up. The association between MS and worsening glucose tolerance remained significant on

multivariate analysis. The only component of MS that was significant for this association was FPG >5.6 mmol/l.

Consensus guidelines on NODAT published in 2005 recommended an OGTT for KTRs with the WHO criteria for IFG (FPG 6.1-6.9 mmol/l) (109). Results from our study show that an even lower FPG level (5.6 mmol/l) is associated with the risk of progression of glucose intolerance. We found this association to be significant in multivariate analysis. Incidentally, this level of FPG is also the ADA criterion for diagnosing IFG and the fasting glucose criterion for MS in the IDF classification.

There were a few limitations to this study. Firstly, this was a retrospective analysis which could not analyse important factors affecting glucose tolerance such as dietary and exercise habits. The wide range of time from transplantation may have contributed to heterogeneity in the risk factors for glucose intolerance but at the same time, the effects of acute rejection and high steroid dose are avoided since patients were studied in the late post-transplant period. However, the results were no different when the time from transplantation was included in the multivariate analysis. Finally, since we did not estimate insulin secretion and sensitivity, it is not possible to define the mechanism of the changes in glucose tolerance in this study.

In conclusion, we have shown that in renal transplant recipients, fasting and post-prandial plasma glucose levels increased over time, leading to worsening glucose tolerance. This occurred in the late post-transplantation period along with increases in BMI and WHR, and despite reductions in steroid and CNI doses. MS was common and was associated with progressive glucose intolerance. MS was particularly useful for identifying patients with a normal OGTT who are likely to progress. Taken together, traditional risk factors for T2DM were found to be associated with abnormal glycaemia late after transplantation with little

apparent influence from immunosuppression parameters. Further studies are needed to determine whether lifestyle interventions in KTRs with MS, especially in those with normal OGTTs, are beneficial in attenuating the progression of glucose intolerance and reducing cardiovascular morbidity.

CHAPTER 5: RESULTS FROM THE STUDY OF HISTORIC VS. RECENT TRANSPLANTATION COHORTS (INCIDENCE STUDY)

5.0 INTRODUCTION

The aims of this study are described in section 1.12 and methods in section 2.3.

The hypotheses being tested in this study are -

1.   Immunosuppression therapy plays a major role in causing glucose metabolism abnormalities early after kidney transplantation.

2.   With modern immunosuppression regimens, the incidence of NODAT is lower compared with older regimens.

3.   Early-diagnosed NODAT exerts a negative impact on patient outcomes.

Therefore the aims of this study were -

a)   To identify the risk factors associated with the development of early (<12 months) NODAT.

b)   To determine the incidence of glucose metabolism abnormalities in a new era of transplantation, and compare it with the incidence in an older era which had a different immunosuppression regimen.

c)   To determine the effect of early-onset NODAT on long-term patient survival.

We have shown in Chapter 3 that tacrolimus plays an important role in the occurrence of NODAT early after renal transplantation, and that older patients are more susceptible to this effect. An important question that arises is whether reducing the exposure to tacrolimus by

lowering the dosage and target blood levels reduces the risk of developing NODAT. We showed in Chapter 4 that measured CNI levels (both tacrolimus and ciclosporin) decreased over time late after transplantation. Despite this, there was an increased prevalence of OGTT abnormalities and NODAT, and the type and blood levels of CNIs were not associated with this increased prevalence. Therefore, the question that still remains is whether decreasing the dosage of tracrolimus and the measured blood levels early after transplantation has any effect on the incidence of NODAT.

In this chapter, we present the results from the historic vs. recent transplantation cohorts study (incidence study). As explained in sections 2.3.3 and 2.3.4, and depicted in Table 2.3, there was an evolution in immunosuppression regimens from the 1997-2001 period (old era) to the 2004-2009 period (new era). We utilised this difference in regimens to conduct a longitudinal, observational cohort study to study the differences in the incidences of IFG and NODAT in two different eras of transplantation. We also analysed the long-term effect of early-diagnosed NODAT.

5.1 DEMOGRAPHIC DATA

Figure 5.1 shows flowcharts depicting the inclusion of patients into this study. The pre-transplant prevalence of DM was 83/362 (23%) in the old era and 116/541 (21%) in the new era (p=0.59). These patients were excluded from the current study. After other exclusions, the old era consisted of 250 patients and the new era 366 patients. All patients included in this study were non-diabetic pre-transplantation. The mean age at transplantation was similar in the two eras (old 45.8±13.7 vs. new 45.3±13.6 years, p=0.60). Also, there was no difference in the BMI or gender distribution between the two eras (Table 5.1). The proportion of patients classified as obese (BMI>30) was similar in both eras (old 21.2% vs. new 21.7%,

p=0.80). Median follow-up time was 49.5 months (range 7-93 months) in the old era and

58.4 months (range 17-100 months) in the new era.

Immunosuppression details are discussed in section 5.5.

Figure 5.1 – Flowchart depicting the inclusion of patients into the incidence study (PNF – primary non-function)
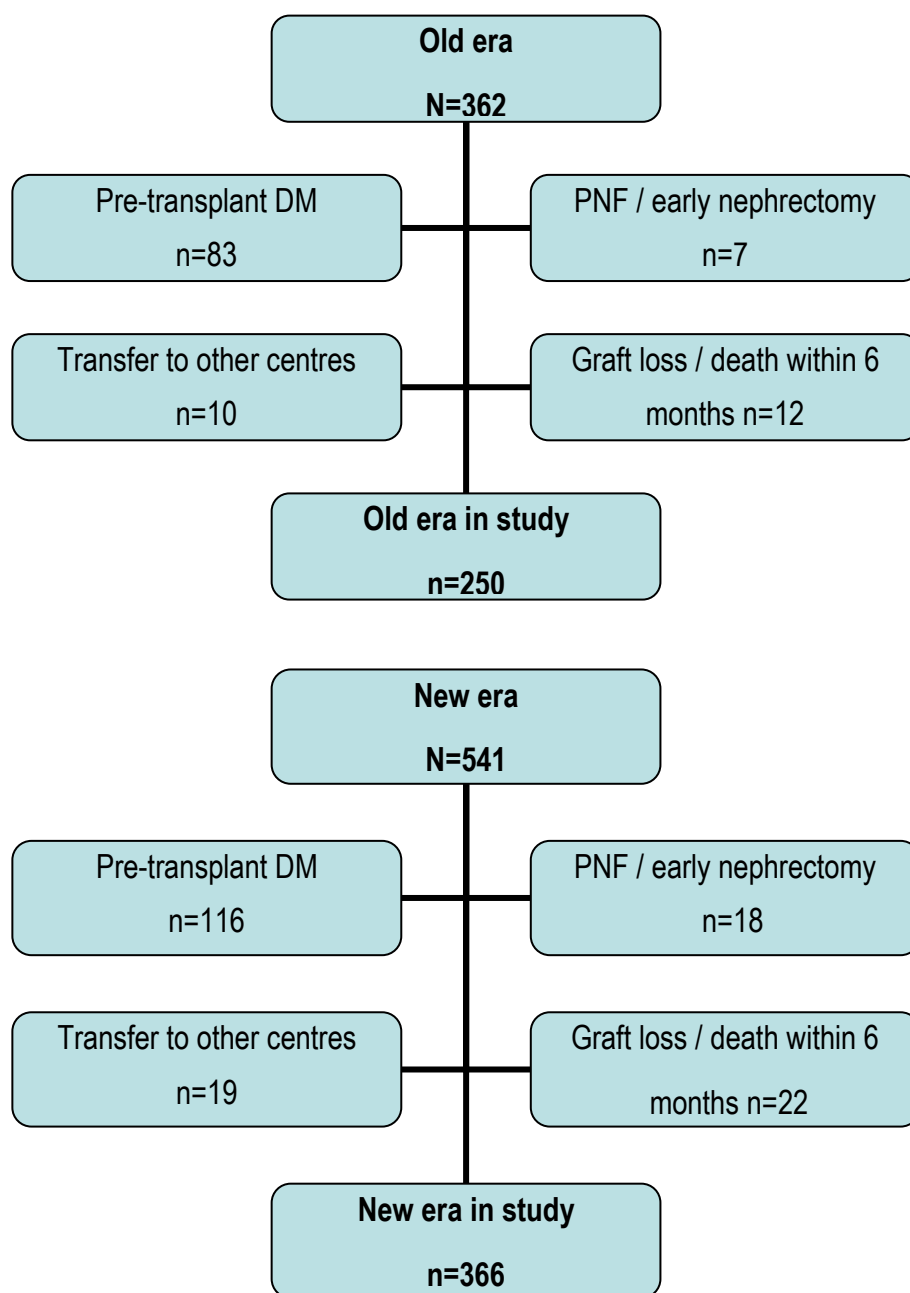
Table 5.1 – Demographic characteristics of patients in the two eras

	Old era n=250	New era n=366	p
Age at transplant in years, mean±1SD	45.8±13.7	45.3±13.6	0.60
Gender, males n (%)	165 (66)	239 (65)	0.80
BMI at transplant in kg/m², mean±1SD	26.1±4.7	26.4±4.6	0.50
BMI > 30 at transplant, n (%)	53 (21%)	79 (22%)	0.80

5.2 INCIDENCES OF IFG AND NODAT IN THE FIRST 12 MONTHS

Figure 5.2 shows the proportions of patients with NFG, IFG and NODAT in the first post-transplant year in the two eras. The incidence of NODAT was significantly lower in the new era (old 47 (19%) vs. new 44 (12%), p=0.01), as was the incidence of IFG (old 49 (20%) vs. 57 (16%), p=0.01). Overall, the proportion of patients who maintained a normal fasting glucose by 12 months was significantly higher in the new era [153 (61%) vs. 265 (72%), p=0.01].

Figure 5.2 - Proportions of patients with normal fasting glucose (NFG), impaired fasting glucose (IFG) and new-onset diabetes (NODAT) by 12 months post-transplantation in the two eras (chi-squared test p=0.01).
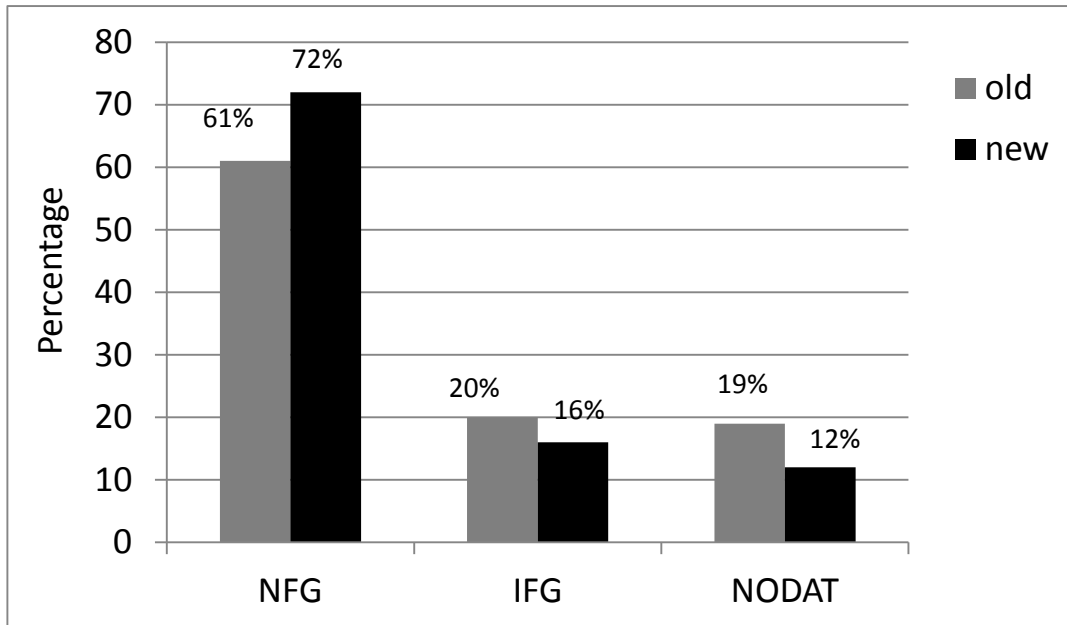
5.3 CUMULATIVE INCIDENCE OF NODAT

The cumulative incidence of NODAT during follow-up was lower in the new era (26% vs. 17%, p=0.007). On Kaplan-Meier analysis, patients in the new era had a significantly longer duration of NODAT-free survival [mean estimate 83.7 months (95% CI 80.1-87.2)] compared to those in the old era [67.4 months (63.2-71.7) (log-rank test p=0.009, Figure 5.3). In both eras, the largest proportion of cases of NODAT occurred within the first 12 months – 71% in the old era and 70% in the new era. The difference in the incidences of NODAT appears to occur within the first year post-transplant. After this period, the incidence curves are almost parallel, indicating comparable rates of NODAT occurrence in both eras. Another

way of depicting the same data is shown in Figure 5.4. This curve was generated using Cox regression analysis, adjusting for age and gender. Figure 5.5 shows the cumulative incidence of NODAT in tacrolimus-treated patients only in both eras. Again, the incidence was lower in the new era.

Overall, 23/63 (36%) patients with NODAT in the new era and 26/66 (39%) in the old era were treated with anti-diabetic medication.

Figure 5.3 – Kaplan-Meier curves of NODAT-free survival for all patients in both eras
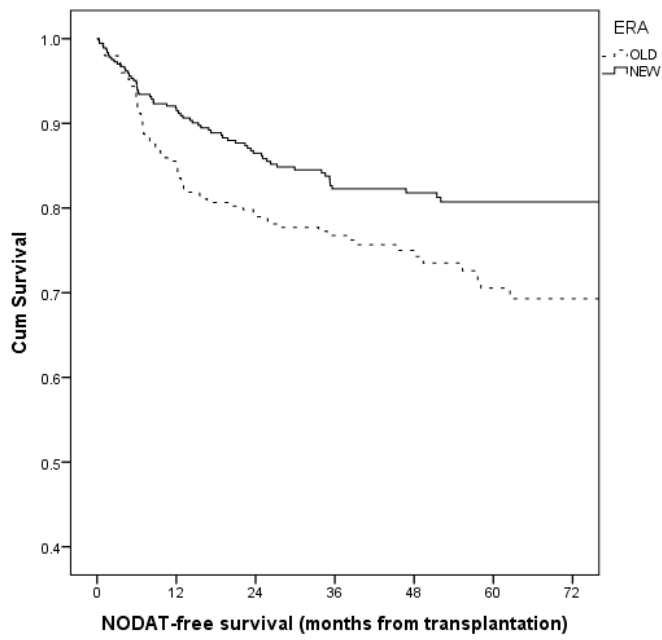
Number at risk →		6 months	12 months	24 months	36 months	48 months	60 months	Total incidence
Old era	At risk, n	250	232	209	172	123	90	
	NODAT incidence, n	18	29	7	5	4	3	**66 (26%)**
New era	At risk, n	366	347	317	280	223	161	
	NODAT incidence, n	19	25	9	7	2	1	**63 (17%)**

Figure 5.4 – Cumulative incidence of NODAT (adjusted) in the two eras - all patients
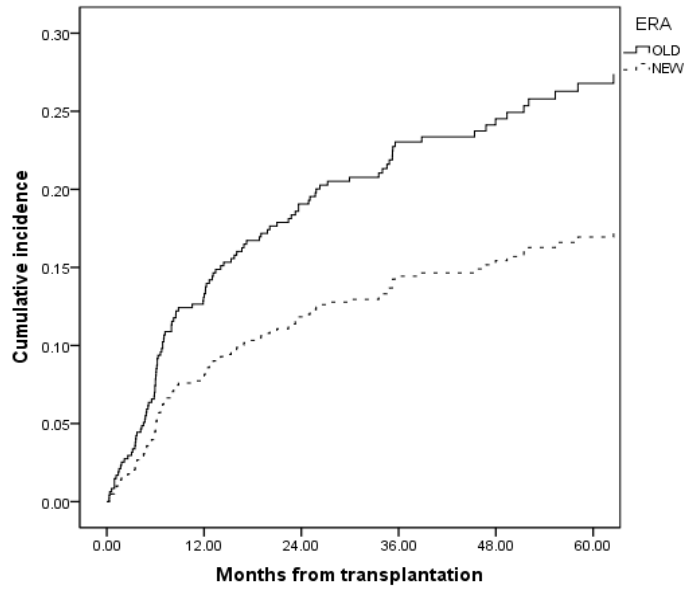
Figure 5.5 – Cumulative incidence of NODAT (adjusted) in the two eras - tacrolimus-treated patients only
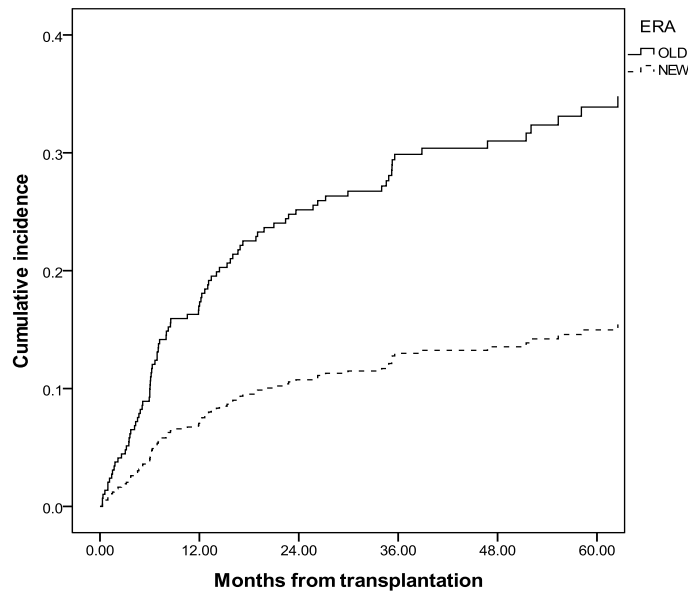
5.4 Incidence of NODAT after the first year post-transplantation

Although the incidence of NODAT within 12 months was higher in the old era, the incidence between 12 and 60 months was similar in both eras [19 (9%) vs. 19 (6%), p=0.16). On Kaplan-Meier analysis also, there was no difference in NODAT-free survival between the two eras when only the incidence after 12 months was considered (log-rank test p=0.26, Figure 5.6).

Figure 5.6 – Kaplan-Meier curves of NODAT-free survival in patients who remained non-diabetic at 12 months after transplantation. Survival time is calculated from 12 months after transplantation (log-rank test p=0.26)
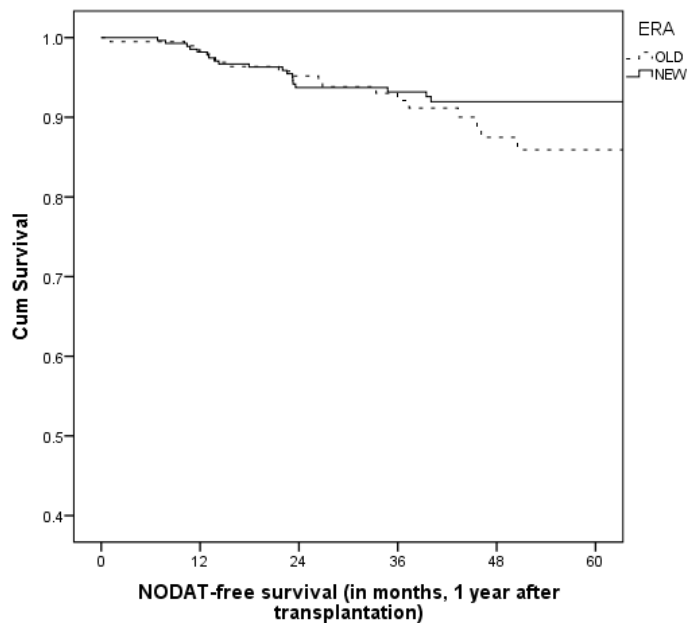
5.5 IMMUNOSUPPRESSION AND REJECTION DATA IN THE TWO ERAS

Table 5.2 summarizes data on immunosuppression and acute rejection. Almost all patients in the new era were initiated on tacrolimus (96%) from the time of transplantation compared with 53% in the old era (p<0.001). Trough tacrolimus blood levels at 6, 12, 36 and 48 months after transplantation were significantly lower in the new era. There was a progressive decrease in the tacrolimus levels over time as shown in Figure 5.7. In the scatter-plots in Figure 5.7, each individual recipient's unique transplant number (increasing consecutively) is taken as a surrogate for time on the X-axis. At all the time-points measured, there was a significant negative correlation between the transplant number (time) and tacrolimus level, as represented by the Pearson correlation coefficient r. When the new era KTRs were analysed separately, the negative trend of tacrolimus level with time was seen from 12 months onwards, and the correlation at 24 months was not significant statistically (Figure 5.8). In these figures, patients with transplant numbers 1854 to 1989 are not included as they were not part of the study.

The proportion of patients on steroid therapy at 12 months post-transplantation was similar in the two eras (old 43% vs. new 46%, p=0.50). The mean cumulative prednisolone dose in the first year after transplantation was 2241 ± 945 mg in the old era compared with 1967 ± 917 mg in the new era (p<0.001). Excluding the intravenous steroid bolus at induction, a higher proportion of patients in the old era had received at least one methylprednisolone dose than those in the new era (34% vs. 25%, p=0.02). When the intravenous steroid bolus given at induction was included in the analysis, KTRs in the old era had received a higher dose of methylprednisolone than those in the new era [median (interquartile range) 500 (1500) vs. 500 (500), p=0.005 by Mann-Whitney U test). In both eras, a significant proportion of

patients who had not suffered a rejection episode were still on steroid therapy at 12 months (old 30% and new 35%, p=0.28).

The incidence of biopsy-proven AR during the whole follow-up was significantly higher in the old era (37% vs. 27%, p=0.008), as was the proportion of AR episodes treated with steroid boluses (old 34% vs. new 25%, p=0.02). Also, the proportion of patients experiencing more than one biopsy-proven AR episode was also higher in the old era compared with the new era (16% vs. 6%, p=0.03).

Table 5.2 – Details of immunosuppression and acute rejection in the two eras

		Old era n=250	New era n=366	p
Primary immunosuppression n (%)	**Ciclosporin**	91 (37)	9 (2)	0.002
	Tacrolimus	133 (53)	350 (96)	
	Sirolimus	26 (10)	7 (2)	
Steroid use at 12 months, n (%)		108 (43)	162 (46)	0.50
Steroid use at 12 months in patients with no AR		46/154 (30%)	90/255 (35%)	0.28
Cumulative prednisolone dose in the first 12 months, mean (SD) mg		2241 (945)	1967 (917)	<0.001
Methylprednisolone use by 12 months, median (interquartile range) mg		500 (1500)	500 (500)	0.004
Number of patients with AR, n (%)		92 (37)	98 (27)	0.006
Number of patients with AR treated with methylprednisolone, n (%)		81 (34)	93 (25)	0.02
Number of patients with 0, 1 or >1 AR episodes, n (%)		0 = 158 (63) 1 = 53 (21) > 1 = 39 (16)	0 = 268 (73) 1 = 78 (21) > 1 = 20 (6)	0.03
Tacrolimus trough level, mean ng/ml (SD)	**6 months**	10.2 (3.3)	8.9 (3.6)	< 0.001
	12 months	9.4 (2.9)	8.4 (3.3)	0.001
	24 months	8.6 (3.0)	8.2 (3.0)	0.12
	36 months	9.0 (3.1)	7.7 (2.5)	<0.001
	48 months	8.9 (3.2)	7.3 (2.6)	<0.001

Figure 5.7 – Scatter-plots of transplant numbers (representing time) versus trough tacrolimus levels in all tacrolimus-treated patients, with Pearson correlation coefficient (r).

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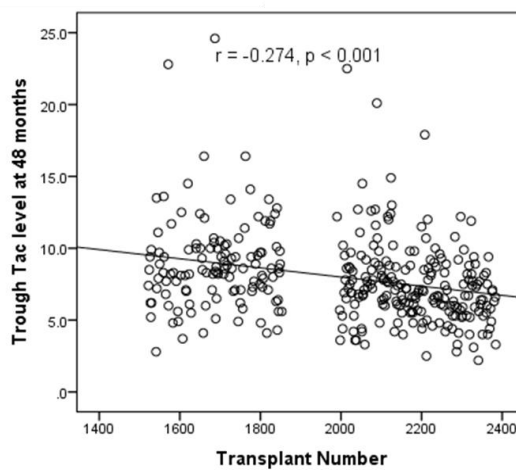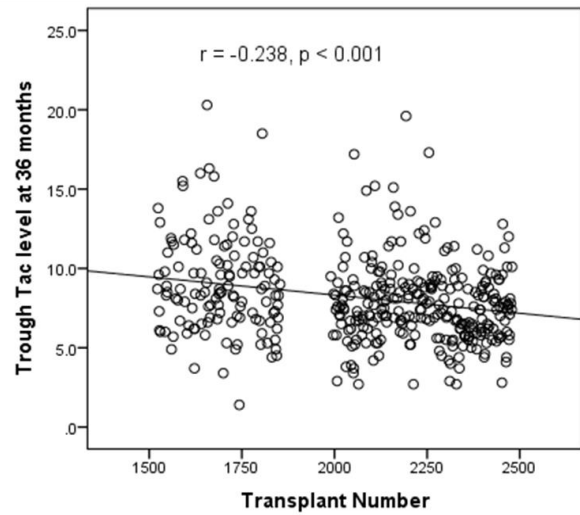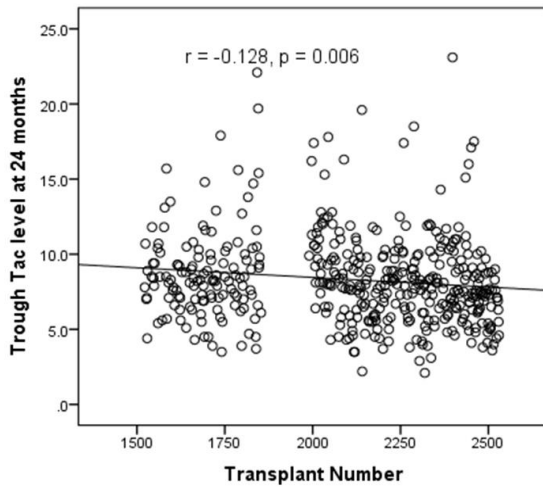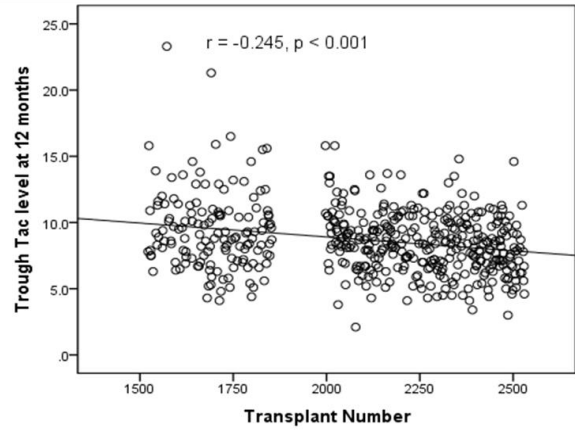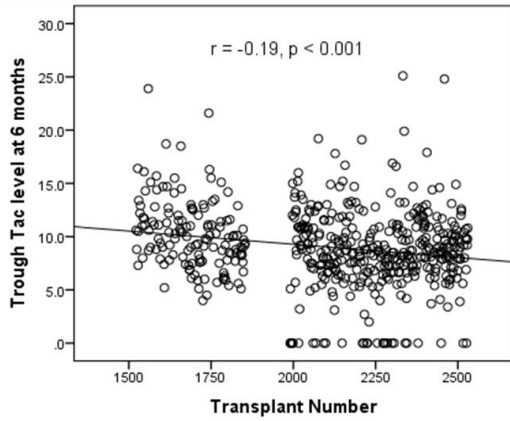
Figure 5.8 – Scatter-plots of transplant numbers (representing time) versus trough tacrolimus levels in tacrolimus-treated patients in the new era only, with Pearson correlation coefficient (r).
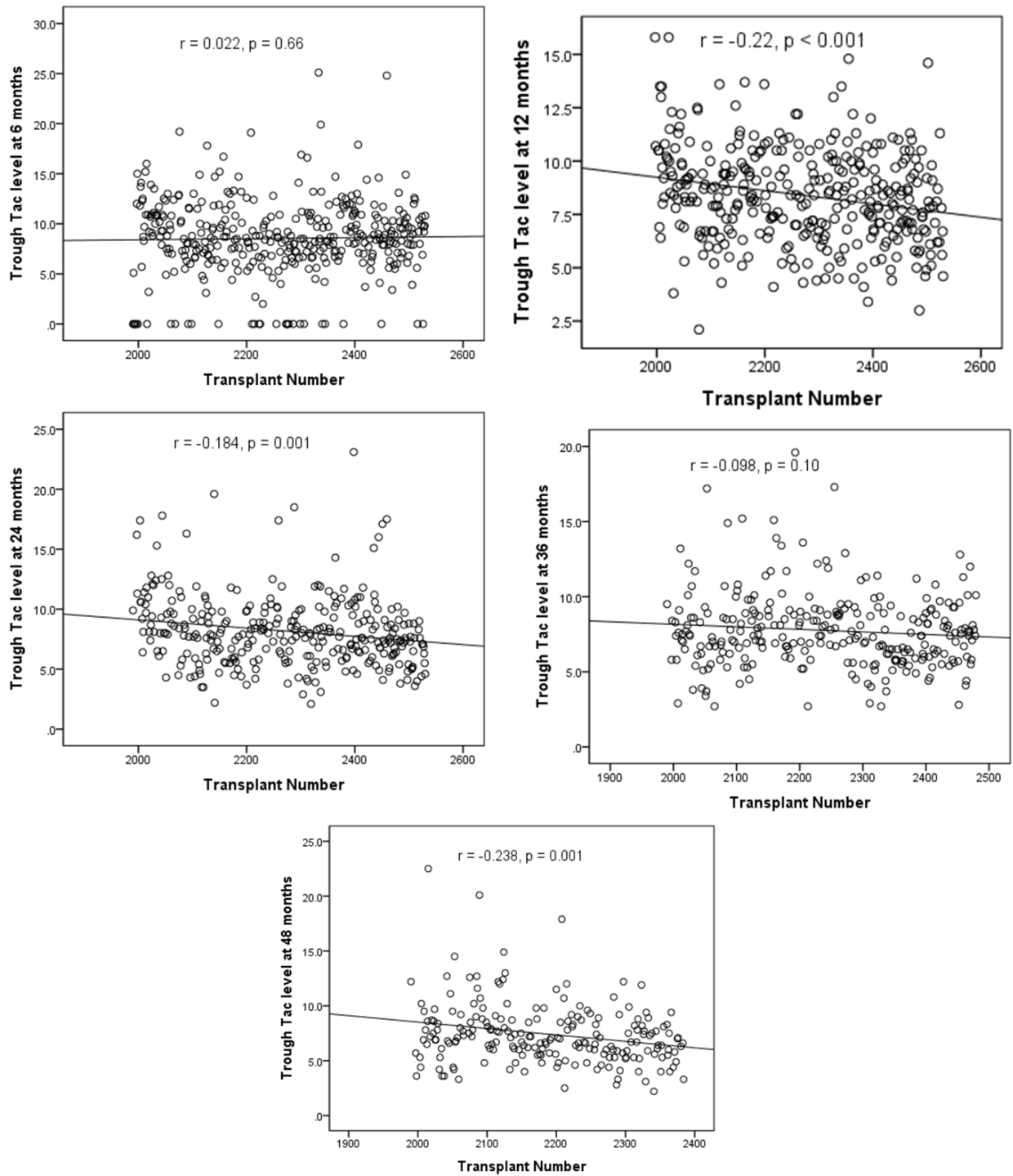
5.5.1 Immunosuppression and rejection data according to NODAT status

As seen in the previous section, there were clear differences in immunosuppression and biopsy-proven AR rates between the two eras. In the old era, patients treated with tacrolimus had a higher 12-month incidence of NODAT than those treated with ciclosporin (based on an intention-to-treat analysis) (26% vs. 12%, $p = 0.01$). We did not perform this analysis for the new era since 96% of patients in this era were treated with tacrolimus.

In an analysis including all tacrolimus-treated patients, mean trough tacrolimus blood levels at 6, 12, 24 and 36 months from transplantation were no different in patients who developed NODAT compared with those who did not (Table 5.3). The results remained similar when the two eras were analysed separately. Despite this finding and 96% of patients in the new era being treated with tacrolimus, we have seen that the incidence of NODAT in the new era was lower than that in the old era.

Table 5.3 – Differences in trough blood tacrolimus levels between NODAT and non-NODAT patients (tacrolimus-treated patients only, N = 483)

Trough blood tacrolimus levels (mean ng/ml ± 1SD	NODAT n = 102	No NODAT n = 381	p
6 months	9.3±3.6	9.2±3.6	0.72
12 months	8.8±2.1	8.6±2.5	0.65
24 months	8.1±2.8	8.3±2.9	0.65
36 months	8.4±3.3	8.0±2.6	0.30

Table 5.4 shows the differences in steroid use between NODAT and non-NODAT patients with both eras included. A lower proportion of KTRs who developed NODAT were on prednisolone therapy at 12 months compared with those who did not develop NODAT (34% vs. 47%, p=0.02). The cumulative prednisolone dose and the mean methylprednisolone dose were similar between the two groups. The results remained similar when the two eras were analyzed separately.

Table 5.4 - Differences in steroid use between NODAT and non-NODAT patients (1st year) (patients from both eras, N=616)

	NODAT n=91	No NODAT n=525	p
Steroid use at 12 months, n	30 (34%)	240 (47%)	0.02
Steroid use at 12 months in patients with no AR	13/61 (21%)	123/348 (35%)	0.04
Cumulative prednisolone dose in the 1st 12 months, mean±SD mg	1951±903	2097±942	0.17
Methylprednisolone use by 12 months, median (interquartile range) mg	500 (1000)	500 (500)	0.62

We next analysed the AR data according to the occurrence of NODAT. For the purpose of this analysis, AR was defined as treatment with at least one dose of methylprednisolone in the context of at least borderline AR on histological examination. Combining both eras, 42 of the 129 (33%) KTRs who developed NODAT had experienced at least one AR episode, as compared to 147 of the 481 (31%) KTRs who did not develop NODAT (p=0.66). This result did not change when the two eras were analysed separately.

We then divided patients according to whether or not they had experienced AR prior to the diagnosis of NODAT. We did this to explore the risk factors which might differ in those with or without AR. Table 5.5 shows BMI, tacrolimus and steroid data for this analysis. Age at transplantation was similar in the sub-groups with and without AR. Although BMI was consistently higher in the sub-group with no AR, this did not reach statistical significance. Blood trough tacrolimus levels were similar in the two sub-groups. However, the cumulative dose of prednisolone in the first 12 months was significantly higher in those with AR compared to those without AR (2774±834 vs. 1604±682, p<0.001).

Table 5.5 – Analysis of BMI, tacrolimus and steroid data in NODAT-AR sub-groups

All data expressed as mean±1SD

	NODAT with no AR, n = 87	NODAT with AR, n = 42	p
Age, years	51±12	52±12	0.87
Baseline BMI, kg/m²	27.9±5	25.9±5	0.12
BMI at 6 months	27.9±5	25.7±4	0.05
BMI at 12 months	28.5±5	27.0±5	0.20
Cumulative prednisolone dose by 12 months, mg	1604±682	2774±834	<0.001
Trough tacrolimus level at 6 months, ng/ml	8.9±3	9.4±5	0.60
Trough tacrolimus level at 12 months, ng/ml	8.7±2	9.3±2	0.27

5.6 VARIABLES AFFECTING THE OCCURRENCE OF NODAT (BOTH ERAS COMBINED)

Logistic regression analysis was performed to identify factors associated with the development of NODAT by 12 months post-transplantation. All factors in the univariate analysis with p values for the odds-ratio <0.1 were entered into a multivariate model. In univariate analysis, age, gender, BMI and era were associated with NODAT (Table 5.6). In multivariate analysis adjusted for age, gender and BMI, being transplanted in the new era was associated with half the odds of developing NODAT compared with the old era (OR 0.509, p=0.008, Table 5.7). Increasing recipient age, male gender and a higher BMI were still significant in multivariate analysis. Interestingly, presence of rejection, cumulative prednisolone dose and the type of CNI were not associated with NODAT even in univariate analysis. As we have seen in the above sections, these three factors were significantly different between the two eras.

Table 5.6 - Logistic regression analysis of factors associated with the development of NODAT within the first year after transplantation (univariate analysis)

Factor	Odds ratio	95% CI	p
Age, per year	1.042	1.024-1.061	<0.001
Gender, male	1.689	1.019-2.797	0.04
Pre-transplant BMI, per unit	1.065	1.017-1.116	0.008
Change in BMI from baseline to 12 months	1.008	0.898-1.131	0.89
Baseline CNI tacrolimus	1.233	0.655-2.322	0.51
AR episode in the 1st year, yes	0.970	0.599-1.570	0.91
Prednisolone therapy at 12 months, yes	1.234	0.581-2.624	0.58
Cumulative 1 year prednisolone dose, grams	0.843	0.661-1.076	0.17
New era	0.574	0.368-0.896	0.01

Table 5.7 - Logistic regression analysis of factors associated with the development of NODAT within the first year after transplantation (multivariate analysis)

Factor	Odds ratio	95% CI	p
Age, per year	1.044	1.023-1.065	<0.001
Gender, male	1.888	1.019-2.797	0.02
Pre-transplant BMI, per unit	1.081	1.017-1.116	0.003
New era	0.509	0.309-0.836	0.008

5.7 VARIABLES AFFECTING THE OCCURRENCE OF NODAT IN TACROLIMUS-TREATED PATIENTS ONLY

A sub-group analysis was performed which included only those KTRs treated with tacrolimus in both the eras. In this model also, on univariate analysis, age, gender, pre-transplant BMI and era were independently associated with the development of NODAT. Again, on multivariate analysis, being transplanted in the new era was associated with a lower risk of developing NODAT in the first year (Table 5.8). In a separate model where "era" was replaced by cumulative prednisolone dose and trough tacrolimus level at six months and twelve months post-transplantation, these three factors were not associated with NODAT.

Table 5.8 – Logistic regression analysis of variables affecting the incidence of NODAT in the first year in tacrolimus-treated patients (multivariate analysis, both eras included)

Factor	Odds ratio	95% CI	p
Age, per year	1.044	1.028-1.061	<0.001
Gender, male	2.110	1.101-4.042	0.02
Pre-transplant BMI, per unit	1.114	1.017-1.116	<0.001
New era	0.312	0.171-0.568	<0.001
[1]Cumulative 1 year prednisolone dose, grams	0.756	0.552-1.036	0.08
[1]6 month trough tacrolimus level	1.005	0.929-1.087	0.90
[1]12 month trough tacrolimus level	1.059	0.937-1.136	0.35

[1] - included in the multivariate model without "era" as a factor

5.8 Characteristics of NODAT patients in the two eras

In this analysis, characteristics of KTRs developing NODAT in the old era were compared with those developing it in the new era (Table 5.9). The mean age of patients developing NODAT in the first year was similar in the two eras (old 51.7 vs. new 51.3 years, p=0.89). The mean BMI in the new era was approximately 1.5 to 2 units higher than that in the old era, although this difference was statistically significant only at 12 months. In keeping with the general trend for all patients, trough tacrolimus levels were lower in the new era at all time points and the cumulative dose of prednisolone was lower in the new era (mean 2193 vs. 1787 mg, p=0.01).

Table 5.9 – Comparison of age, BMI, tacrolimus levels and steroid exposure between NODAT patients in the old and new era

	NODAT in old era n = 47	NODAT in new era n = 44	p
Age in years, mean (SD)	51.7 (13)	51.3 (11)	0.89
BMI in kg/m², mean (SD)			
Baseline	26.5 (4.9)	28.1 (4.9)	0.12
6 months	26.5 (4.9)	28.2 (4.5)	0.11
12 months	27.1 (4.9)	29.1 (4.5)	0.05
24 months	28.1 (4.7)	29.7 (5.2)	0.18
36 months	27.9 (5.2)	29.0 (4.6)	0.40
48 months	27.3 (5.3)	28.9 (5.1)	0.30
Change in BMI from pre-transplant to 12 months	0.64 (1.9)	0.83 (2.7)	0.73
Trough tacrolimus level in ng/ml, mean (SD)			
6 months	10.7 (3.7)	7.9 (3.5)	0.001
12 months	9.2 (1.9)	8.6 (2.2)	0.19
24 months	8.7 (2.8)	7.9 (2.6)	0.25
36 months	9.5 (3.9)	7.0 (1.3)	0.003
48 months	8.0 (2.0)	6.8 (2.4)	0.05
Cumulative prednisolone use in the 1st year, mean (SD) mg	2193 (993)	1787 (868)	0.01

5.9 CHARACTERISTICS OF EARLY- AND LATE-ONSET NODAT PATIENTS

Since the incidence of NODAT was highest in the first 12 months in both eras, we examined for differences between patients developing NODAT within 12 months and those developing it after 12 months. There was no difference in age, gender or BMI distributions between these two groups of patients. Also, acute rejection rates, tacrolimus use, trough tacrolimus levels and cumulative prednisolone use in the first year were similar between the two groups (Table 5.10).

Table 5.10 – Characteristics of patients developing NODAT before and those developing after 12 months of transplantation (two eras combined)

		NODAT within 12 months n=91	NODAT after 12 months n=38	p
Age at transplant, mean (SD)		51.7 (12)	49.5 (11)	0.33
Gender, males n (%)		68 (75)	25 (66)	0.30
BMI at transplant, mean (SD)		27.4 (5)	27.4 (3.5)	0.90
BMI at 12 months, mean (SD)		28.0 (4.8)	28.1 (4.5)	0.90
Tacrolimus treated, n (%)		74 (81)	28 (74)	0.60
Acute rejection, n (%)		29 (32)	14 (37)	0.68
Tacrolimus trough level, mean ng/ml (SD)	6 months	9.1 (3.8)	8.4 (4.5)	0.42
	12 months	8.9 (2.1)	8.5 (2.2)	0.37
Cumulative prednisolone use in the 1st year, mean (SD) mg		1951 (903)	2074 (1104)	0.51

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5.10 NODAT AND EFFECT ON OUTCOMES

In this section, we examine the effect of NODAT diagnosed within the first year after transplantation on graft and patient outcomes.

5.10.1 EFFECT ON DEATH-CENSORED GRAFT SURVIVAL (DCGS)

Overall, a diagnosis of NODAT in the first year after transplantation did not have a negative effect on DCGS on Kaplan-Meier analysis (NODAT 94.6% vs. no NODAT 94.3%, p=0.9). The results did not change when analyzed separately for old and new eras (Figures 5.9a, 5.9b and 5.9c).

Figure 5.9a – DCGS in patients with and without NODAT (both eras)
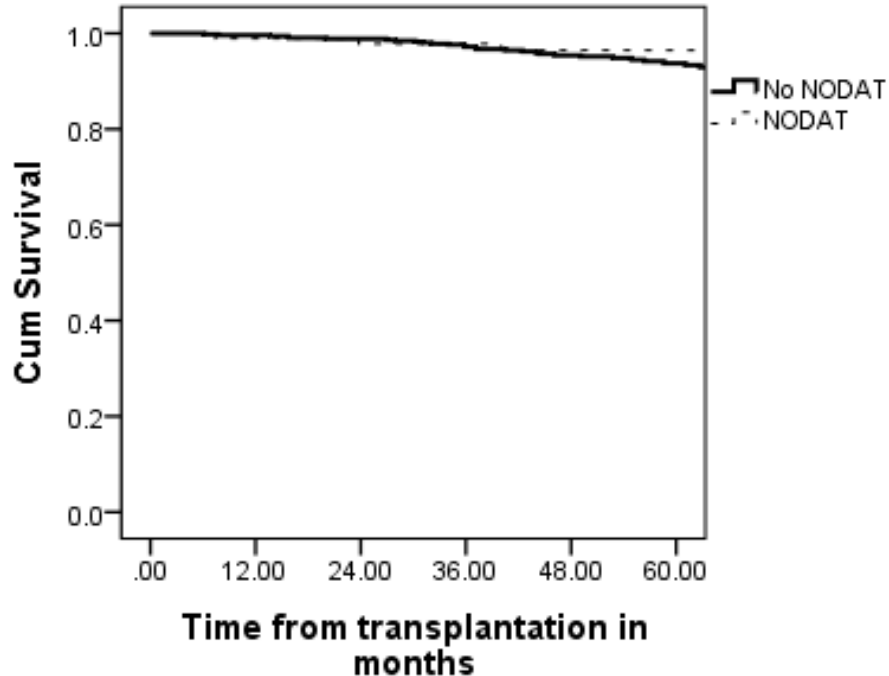
Figure 5.9b – DCGS in patients with and without NODAT (old era only)
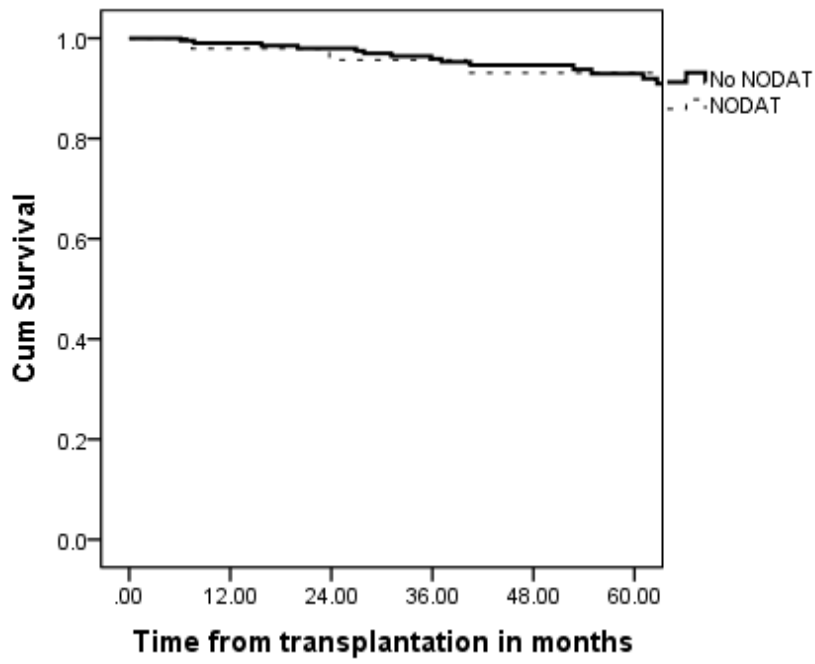
Figure 5.9c– DCGS in patients with and without NODAT (new era only)
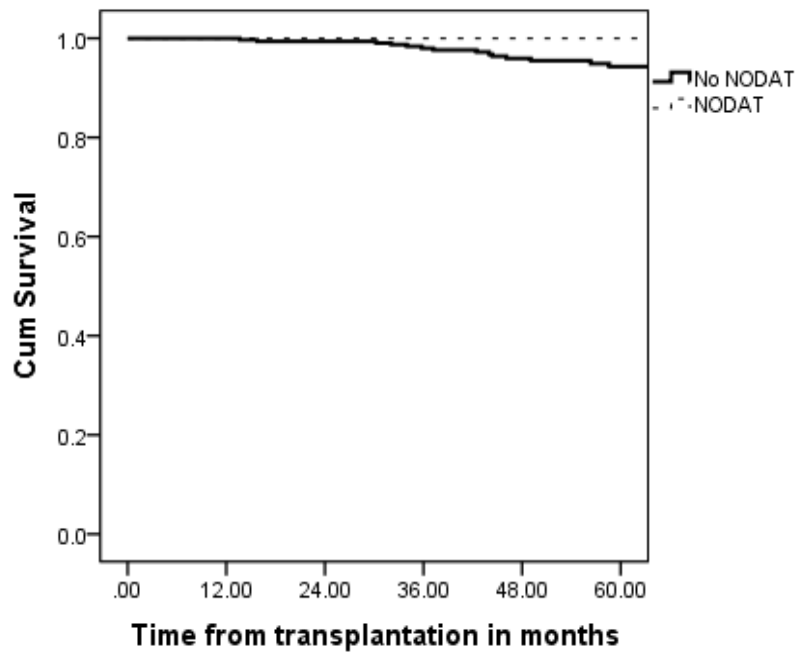
5.10.2 Effect of NODAT on patient survival – univariate analysis

During the follow-up period, a total of 41 patients died - 18 transplanted in the old era and 23 from the new era. There was no difference in the overall probability of patient survival (all patients) between the two eras (old era 92% vs. new era 95%, log-rank test p=0.17, Figure 5.10).

Figure 5.10 –Patient survival according to the era of transplantation
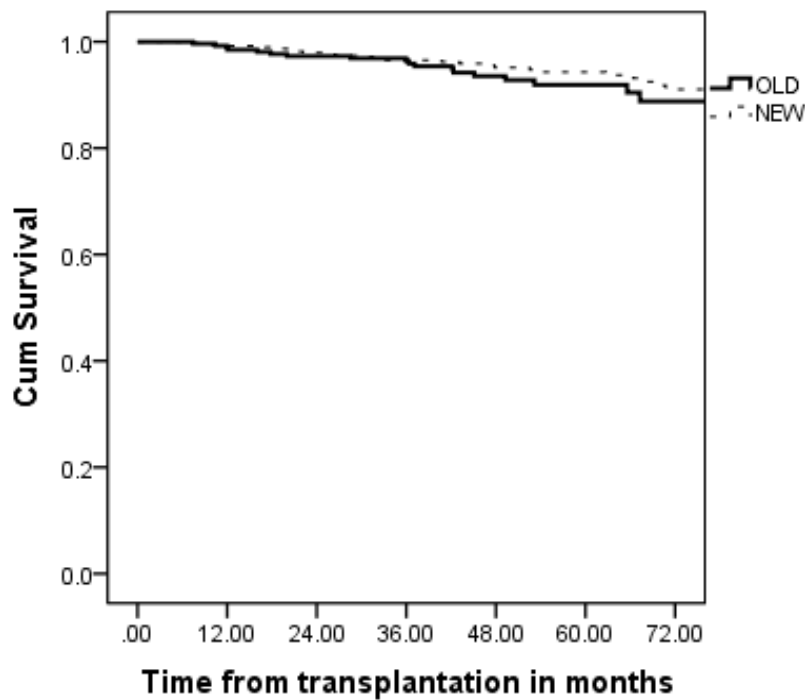
Overall, a diagnosis of NODAT in the first year after transplantation significantly reduced the probability of 5-year patient survival (No NODAT 97% vs. NODAT 87%, log-rank test p=0.002, Figure 5.11a). The adverse effect of NODAT on patient survival was seen in both eras, as shown in Figures 5.11b and 11c. Five-year patient survival estimations according to the era and NODAT status are shown in Table 5.11 (log-rank test p=0.005).

Table 5.11 – Five-year estimated patient survival rates according to era and NODAT status (Kaplan-Meier method)

Old era, no NODAT	94%
Old era, NODAT	85%
New era, no NODAT	96%
New era, NODAT	89%

Figure 5.11a –Patient survival according to the presence of NODAT (both eras)
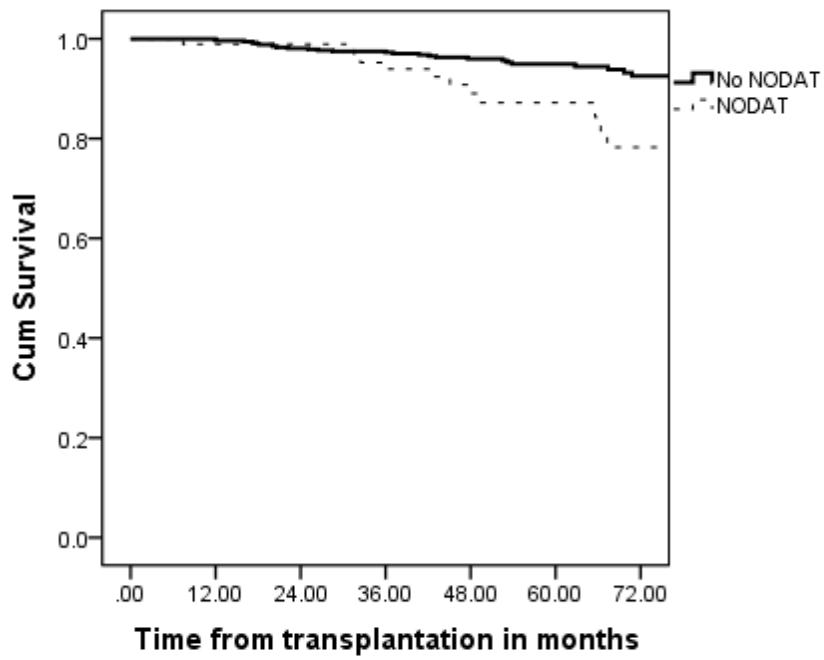
Figure 5.11b –Patient survival according to the presence of NODAT (old era only)
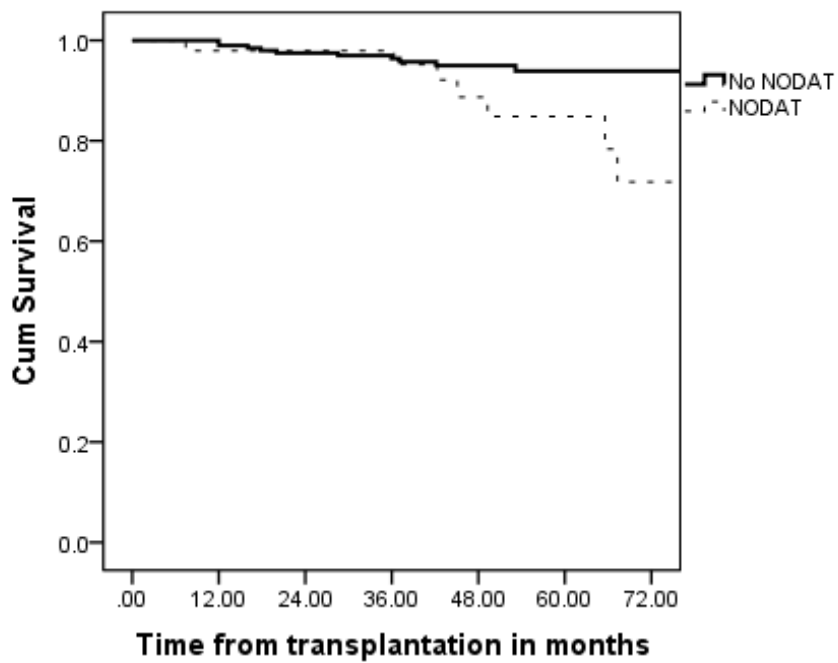
Figure 5.11c –Patient survival according to the presence of NODAT (new era only)
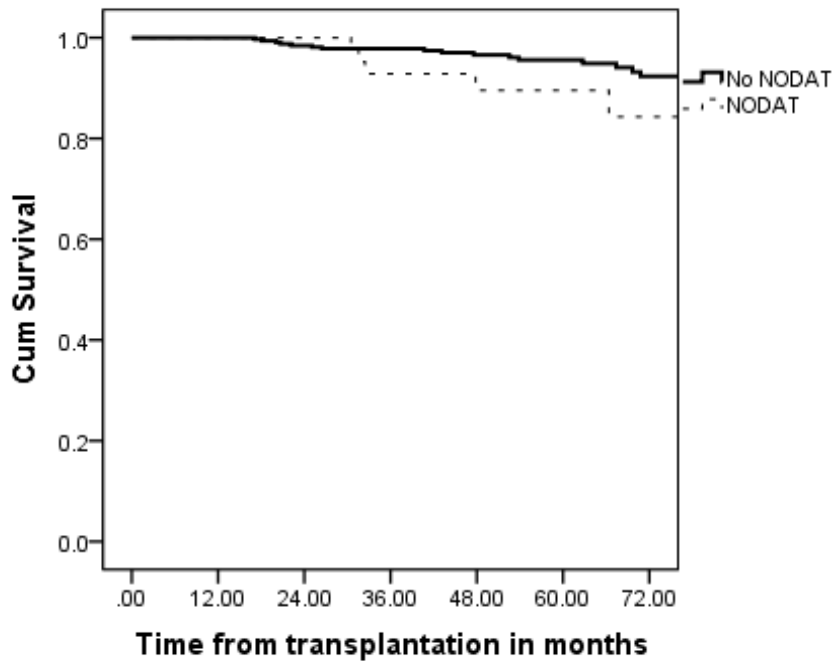
5.10.3 EFFECT OF NODAT ON PATIENT SURVIVAL – MULTIVARIATE ANALYSIS

On Cox's regression analysis, after correcting for age, gender and the era of transplantation, patients with NODAT diagnosed within one year had a higher risk of death than those without NODAT (HR 1.76, Table 5.12a). In a separate multivariate model, when NODAT was replaced by FPG at one year, increasing FPG levels were associated with a higher risk of death (HR 1.26, Table 5.12b). A higher 12-month S. creatinine (both models) and higher systolic BP (model 2) were also associated with increased mortality.

Table 5.12a- Cox's proportional hazards regression model for all-cause mortality (all factors with a hazard ratio p value of <0.1 in the univariate analysis were entered into the multivariate analysis)

	Univariate			Multivariate model 1		
	Hazard ratio	95% CI	p	Hazard ratio	95% CI	p
Recipient age (per year)	1.05	1.02-1.07	<0.001	1.05	1.01-1.09	0.005
NODAT in 1 year (yes)	2.77	1.44-5.36	0.002	1.76	1.16-4.95	0.04
S.creatinine (per µmol/l)	1.01	1.006-1.01	<0.001	1.01	1.003-1.01	0.003
FPG at 12 months (permmo/l)	1.25	1.03-1.50	0.02	-		
AR episodes yes	1.68	0.90-3.12	0.09	1.96	0.82-4.69	0.12
Systolic BP at 12 months (per 5mmHg)	1.13	1.05-1.21	0.001	1.04	0.94-1.15	0.10
Gender male	0.79	0.42-1.81	0.46			
Prednisolone use at 12 months (yes)	1.11	0.60-2.05	0.74			
Pre-transplant BMI	0.97	0.90-1.04	0.37			
BMI at 12 months	0.98	0.91-1.05	0.54			
Baseline CNI tacrolimus	0.85	0.39-1.85	0.68			
Era, new	0.62	0.33-1.17	0.14			
Cumulative prednisolone use (per 100mg)	1.20	0.87-1.67	0.27			
Diastolic BP at 12 months (per 5mmHg)	1.07	0.92-1.25	0.36			
S. total cholesterol at 12 months	1.06	0.81-1.37	0.69			
S. triglycerides at 12 months	1.08	0.87-1.34	0.47			

Table 5.12b - Cox multivariate regression analysis of variables associated with all-cause mortality (model 2 – NODAT in the 1st year replaced by FPG at one year)

	Univariate			Multivariate model 2		
	Hazard ratio	95% CI	p	Hazard ratio	95% CI	P
Recipient age (per year)	1.05	1.02-1.07	<0.001	1.04	1.01-1.07	0.003
NODAT in 1 year (yes)	2.77	1.44-5.36	0.002	-		
S. creatinine (per µmol/l)	1.01	1.006-1.01	<0.001	1.009	1.002-1.010	0.01
FPG at 12 months (per mmol/l)	1.25	1.03-1.50	0.02	1.26	1.03-1.54	0.02
AR episodes yes	1.68	0.90-3.12	0.09	1.78	0.89-3.59	0.10
Systolic BP at 12 months (per 5mmHg)	1.13	1.05-1.21	0.001	1.08	1.001-1.169	0.04

5.11 ANALYSIS OF CARDIOVASCULAR RISK FACTORS

Table 5.13 shows the comparison of CV risk factors between NODAT and non-diabetic KTRs with data for both eras combined. Patients with NODAT consistently had higher values of BMI, systolic BP, diastolic BP and S. triglycerides, at least until 36 months after transplantation. Serum total cholesterol was no different between the two groups at any time point.

There was a significant positive correlation between BMI and systolic BP, diastolic BP and S. triglycerides at 12 months (Table 5.14), but not with S. total cholesterol or the type of primary immunosuppressive agent.

Table 5.13 – Comparison of cardiovascular risk factors in patients with and without NODAT in the first year after transplantation [mean (SD)] – both eras combined

	NODAT n=102	No NODAT n=381	p
Baseline BMI, in kg/m²	27.4 (4.9)	25.8 (4.7)	0.007
BMI at 6 months	27.4 (4.7)	26.0 (4.6)	0.016
BMI at 12 months	28.0 (4.8)	26.5 (4.8)	0.015
BMI at 24 months	28.9 (5.0)	27.1 (5.2)	0.009
BMI at 36 months	28.3 (5.0)	27.2 (5.4)	0.13
BMI at 48 months	28.0 (5.2)	27.5 (5.6)	0.60
Systolic BP at 6 months, in mmHg	149 (27)	141 (20)	0.01
Systolic BP at 12 months	147 (21)	139 (20)	0.004
Systolic BP at 24 months	149 (22)	141 (20)	0.004
Systolic BP at 36 months	149 (25)	145 (23)	0.70
Systolic BP at 48 months	150 (24)	139 (19)	0.001
Diastolic BP at 6 months, in mmHg	82 (12)	79 (11)	0.07
Diastolic BP at 12 months	82 (12)	79 (11)	0.03
Diastolic BP at 24 months	82 (11)	79(10)	0.02
Diastolic BP at 36 months	79 (12)	79 (11)	0.90
Diastolic BP at 48 months	80 (13)	79 (11)	0.94

Table 5.13 continued – Comparison of cardiovascular risk factors in patients with and without NODAT in the first year after transplantation [mean (SD)]

	NODAT n=102	No NODAT n=381	p
S. total cholesterol at 6 months, in mmol/l	5.1 (1.4)	5.0 (1.1)	0.45
S. total cholesterol at 12 months	4.8 (1.2)	4.8 (1.2)	0.98
S. total cholesterol at 24 months	4.6 (0.9)	4.7 (1.0)	0.53
S. total cholesterol at 36 months	4.6 (1.1)	4.6 (0.9)	0.69
S. total cholesterol at 48 months	4.6 (0.9)	4.5 (0.8)	0.80
S. triglycerides at 6 months, in mmol/l	2.2 (1.2)	1.8 (1.3)	0.03
S. triglycerides at 12 months	2.1 (1.0)	1.7 (1.1)	0.008
S. triglycerides at 24 months	2.1 (1.3)	1.6 (1.1)	<0.001
S. triglycerides at 36 months	1.9 (0.9)	1.6 (0.9)	0.04
S. triglycerides at 48 months	3.0 (1.5)	1.5 (0.8)	0.03

Table 5.14 – Correlation between BMI and other cardiovascular risk factors at 12 months post-transplantation (both eras combined)

BMI versus ↓	Pearson correlation coefficient	p
Age	0.02	0.53
Systolic BP	0.12	0.007
Diastolic BP	0.13	0.003
S. total cholesterol	0.01	0.76
S. triglycerides	0.17	<0.001
	Spearman's rho	
Primary immunosuppressive agent	0.008	0.85
Cumulative prednisolone dose	-0.06	0.14

5.12 DISCUSSION

In this study, the main finding was a reduction in the cumulative incidence of NODAT in KTRs treated with newer immunosuppression protocols compared with those treated with older protocols. This reduction was seen despite similar age, gender and BMI distributions, and a higher proportion of patients treated with tacrolimus in the new era. At the same time, acute rejection rate, trough tacrolimus levels and cumulative prednisolone use were lower in the new era. However, on multivariate analysis, cumulative prednisolone dose and tacrolimus levels were not associated with NODAT development. The difference in NODAT incidence between the two eras was largely due to a difference in incidence in the first 12 months post-transplantation, since there was no significant difference in incidence after 12 months between the two eras (Figure 5.4). NODAT diagnosed within one year of transplantation did not adversely affect graft survival but was associated with an increased risk of all-cause mortality starting from about three years post-transplantation. Patients with NODAT were noted to have worse cardiovascular factors compared to those without NODAT.

In this study, the 12-month incidence of NODAT in those transplanted between 1997 and 2001 was 19%. This incidence figure is higher than that reported by other studies during that era. For example, Cosio et al. reported an incidence of 10.6% in patients transplanted between 1995 and 1999 (125), and Sakhuja et al. reported an incidence of 5.4% in 1995 (213). However, none of the patients in these two single-centre studies received tacrolimus. Other studies during the late 1990s which included patients treated with tacrolimus were reporting incidences of NODAT between 14% and 20% (199, 200), figures comparable to our study.

In the old era, the 12-month incidence of NODAT of 26% amongst KTRs receiving tacrolimus was significantly higher than the 12% incidence amongst patients receiving ciclosporin. This finding is consistent with several other studies which have reported a higher incidence of NODAT in tacrolimus-treated patients compared to cicosporin-treated patients (156, 199-201). This was confirmed in the randomised and controlled DIRECT study in which all patients received MMF, steroids and basiliximab, and either tacrolimus or ciclosporin (156). In the DIRECT study, the 6-month incidence of NODAT / IFG was significantly higher in the tacrolimus arm (33.6%) compared with the ciclosporin arm (26%, p=0.046). The target trough tacrolimus levels were 10-15 ng/ml for months 1-3, and 5-10 ng/ml subsequently, identical to the targets in the old era in our study.

In the randomised and controlled ELITE-Symphony study, target trough levels in the low-dose tacrolimus arm was 3-7 ng/ml from the time of transplantation (157). This group was compared against patients randomised to standard-dose or low-dose ciclosporin and low-dose sirolimus. All patients in this study were given prednisolone 20 mg daily from the time of transplantation and tapered down to at least 5 mg daily from month four. The incidence of NODAT at 12 months was 8.4% in the low-dose tacrolimus group compared with 6% in the standard-dose ciclosporin and 4.2% in the low-dose ciclosporin groups (log-rank test p=0.02). These target levels and incidences of NODAT (tacrolimus-treated patients) are comparable to that in the new era in our study. The higher incidence of NODAT in the low-dose tacrolimus group in the ELITE-Symphony study was seen in the context of patients in this group experiencing a lower rate of biopsy proven AR, and suspected and treated AR episodes compared to the other groups. Likewise in our study, the rate of AR was significantly lower in the new era, as was the cumulative steroid exposure in the first year.

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In vitro studies have evaluated the mechanisms by which CNIs predispose to developing diabetes. As we have seen in Chapter 1, there is evidence that insulin gene transcription is mediated through NFAT via calcineurin-dependent pathways in pancreatic β-cells (26, 214), and that this action is inhibited by tacrolimus and ciclosporin (215). There is also some evidence in human studies that this β-cell toxicity of tacrolimus is dose-dependent. In one prospective study, reducing tacrolimus blood levels by 30% within therapeutic levels increased insulin secretion by 24% as determined by the intravenous glucose tolerance test (216). In our centre, the starting dose of tacrolimus has progressively decreased with time. More importantly from the point of view of diabetogenecity, the actual delivered dose of tacrolimus has also decreased, as suggested by lower trough levels in the new era compared with the old era (Table 5.2 and Figure 5.7). Even within the new era, there has been a progressive fall in the actual trough tacrolimus levels (Figure 5.8). Therefore, one of the possible reasons for less NODAT in the new era in our study could be less β-cell toxicity consequent to lower tacrolimus exposure. However, in regression analysis, we were not able to show an association between tacrolimus levels and NODAT (Table 5.8).

We have also demonstrated that steroid use was significantly lower in the new era. The total actual delivered doses of both methylprednisolone and prednisolone in the first year after transplantation were lower in the new era. This decrease was seen in the context of less biopsy proven AR episodes and less AR episodes treated with steroid boluses in the new era (Table 5.2). Use of induction immunosuppression therapy followed by tacrolimus has led to a decrease in AR rates which has allowed an earlier tapering of prednisolone and a consequent lower cumulative dose of steroids. Therefore, the other possible reason for less NODAT in the new era is lower steroid exposure leading to a less intense adverse effect on insulin

sensitivity. Again, we were not able to show this association in the regression analysis (Table 5.6).

The risk factors for NODAT identified in this study were old era, increasing age, higher BMI and male gender. Interestingly, on regression analysis, cumulative prednisolone dose, the type of CNI and trough tacrolimus levels were not associated with the development of NODAT. Nevertheless, these factors along with the rate of rejection were significantly lower in the new era. Since being transplanted in the new era was clearly associated with a decreased risk of NODAT on multivariate analysis, we speculate that there were other unmeasured factors that may have been different between the two eras which contributed to the differential risk of NODAT. These factors might include a family history of DM and CMV infection. We did not have data on the family history of DM. Management of CMV was different in the two eras in terms of prophylaxis, diagnosis and treatment. Prophylaxis was used regularly only in the new era. In both eras, testing for CMV was undertaken in the event of clinical suspicion, not as a routine. Diagnosis was by serological means in the old era whilst DNA polymerase chain reaction is used currently. Due to these significant differences, it would not be possible to accurately compare the true rate of CMV infection between the two eras as this was an observational study. Moreover, the temporal relationship between identifying CMV infection and diagnosing NODAT would also be difficult to establish. Due to these complexities, we did not include CMV infection in the logistic regression model for identifying risk factors for NODAT. Nevertheless, there is evidence from one single-centre retrospective study that the incidence of CMV infection is decreasing (127). This was despite closer screening and using a more sensitive diagnostic test in a more recent cohort of KTRs.

The secondary immunosuppressive agents used in our centre (azathioprine or MMF) are not associated with an increased risk of NODAT. This was shown in a meta-analysis by Heisel et al. in 2004 (53). However, there was not enough detail in the original studies to determine this conclusively. Also, one retrospective study comprising of 264 KTRs found an association between basiliximab and an increased incidence of glucose tolerance abnormalities at ten weeks post-transplantation (217). In this study by Aasebo et al., 51.5% of patients who received basiliximab developed NODAT, IGT or IFG, compared with 36.9% of those who did not receive the drug (p=0.017). However, there were several limitations to this study including a higher BMI and a higher rate of switching from ciclosporin to everolimus in the basiliximab group. This finding has not been confirmed in a prospective study.

The rate of biopsy-proven AR (including borderline) and the proportion of patients with multiple AR episodes were both significantly lower in the new era (Table 5.2). This difference is most likely due to the different immunosuppression protocols employed in the two eras. While no induction therapy was used in the old era, almost all patients received induction therapy with basiliximab or ATG in the new era. Standard maintenance therapy was with tacrolimus and MMF in the new era. Tacrolimus has been shown to be associated with lower rates of AR compared with ciclosporin, both in combination with azathioprine (218) and MMF (157).

The association of BMI with NODAT was clearly seen in this study. A high baseline BMI was an independent predictor of the development of NODAT. This was seen in both eras. An important observation was that the baseline BMI of all patients was similar in the two eras (means 26.1 and 26.4, proportions with BMI>30 were 21.2% and 21.7%). However, when the BMIs of only those patients developing NODAT were compared between the two eras, it

was observed that the mean BMI of patients from the new era was consistently 1.5-2 units higher than those from the old era. This difference was significant statistically at 12 months post-transplantation. This observation must be viewed in the context of the degree of tacrolimus and steroid exposures (Table 5.8). Trough tacrolimus levels and cumulative prednisolone use were significantly lower in the NODAT patients from the new era compared to those from the old era. These observations can be graphically represented as shown in Figure 5.12. The inference is that in the new era, recipients who were potentially going to develop NODAT already had lower insulin sensitivity due to their higher BMIs. Consequently, a lower degree of insulin secretory defect (as deduced from lower tacrolimus dose and levels) was enough to render them overtly hyperglycaemic.

A further sub-analysis which we performed also demonstrates the relationship between BMI, the level of immunosuppression and the risk of NODAT (Table 5.5). The BMI of patients who were treated for AR and subsequently developed NODAT, was lower than those who developed NODAT without having suffered an AR episode. Unsurprisingly, cumulative steroid use was higher in those with AR, but the trough tacrolimus levels were not significantly different. The inference from this sub-analysis is that the higher steroid exposure caused a larger negative effect on insulin sensitivity in those with AR, and the reduction in insulin secretion from β-cell toxicity of CNIs caused a decompensation of glycaemic regulation.

Figure 5.12 – Graphical representation of the distribution of BMI and the risk of NODAT
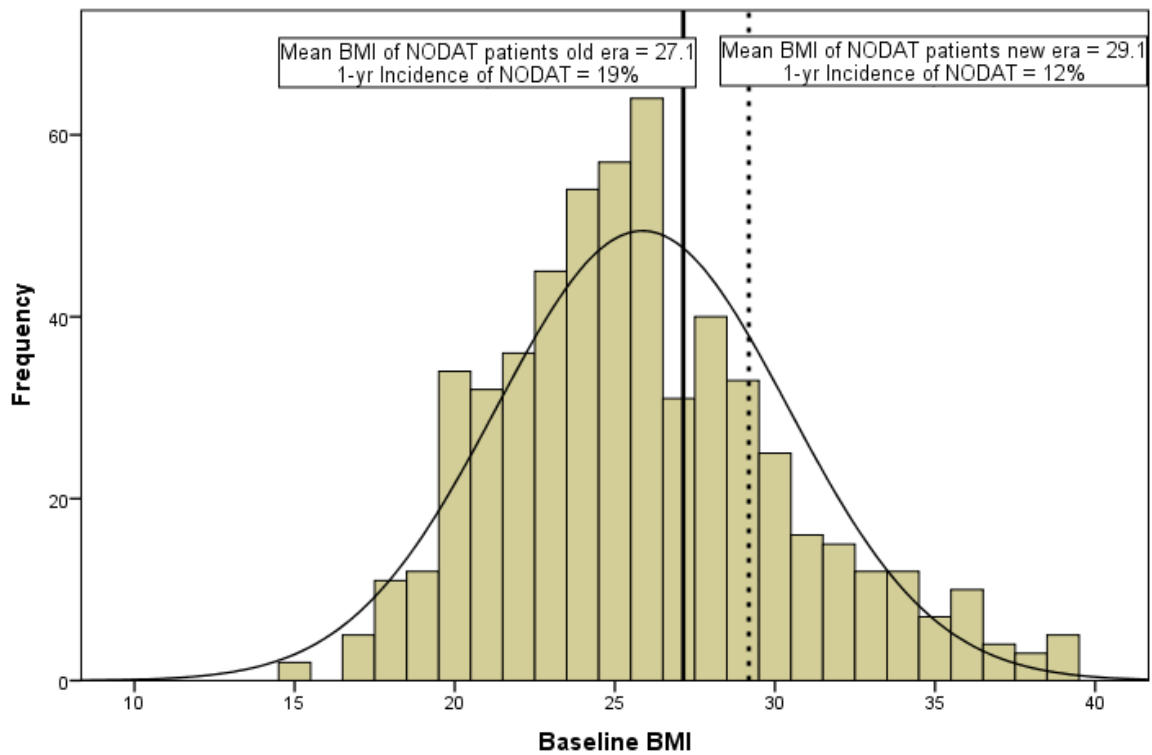
An interesting finding in this study was that although there was a higher incidence of NODAT in the old era compared with the incidence in the new era within 12 months post-transplantation, incidences after 12 months were the similar in the two eras (Figure 5.4). However, the yearly incidence of 2-3% after 12 months in the old era was still, in general, higher than the estimated incidence of T2DM in the UK general population for the same time period (31). On the other hand, the yearly incidence of 0-2% of NODAT in the new era was comparable to the general population T2DM incidence of approximately 0.5% for the same time period. It seems plausible that when CNI doses and blood levels are decreased after the first few months post-transplantation, traditional risk factors for T2DM such as age, high

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BMI and family history play a more significant part in the causation of diabetes, and the incidence rate decreases towards the general population figures. Nevertheless, the presence of CNIs which have an adverse effect on β-cells may still play a significant role in KTRs, by preventing an increase in insulin secretory response when necessary. Of course, an ascertainment bias due to more regular blood tests in KTRs could also lead to a higher incidence in this group of patients.

We could not identify any differences in characteristics between those recipients who developed NODAT before 12 months and those who developed it after 12 months. Vigilance is required even late after transplantation to identify patients with hyperglycaemia in a timely manner.

The adverse effect of early diagnosed NODAT on mortality was seen again in the incidence study, as seen earlier in the Cic vs. Tac study results in Chapter 3. The hazard ratio for all-cause mortality in those KTRs who developed NODAT within the first year was 1.76, after adjusting for age, gender and era of transplantation (Table 5.12a). Although the incidence of NODAT itself was lower in the new era, its adverse effect on mortality occurred in both eras (Figure 5.11b and 5.11c). These results confirm the findings from previous cohort studies which have shown an association between NODAT and poor patient survival (113, 115, 116). These studies were from an era which corresponds to the old era in this study and there is little literature studying the effect of NODAT in the modern era of immunosuppression.

We have shown in this study that higher fasting plasma glucose levels in KTRs are associated with increased mortality. This effect was independent of age, BP, fasting lipids, BMI and S. creatinine (Tables 5.12a and b). Amongst those diagnosed with NODAT, 36% of patients in the new era and 39% in the old era were treated with anti-diabetic medication. Previous

studies have shown the adverse effect of increasing FPG levels on cardiovascular events in KTRs. For example, in a study by Cosio et al., KTRs transplanted between 1998 and 2003 were studied. After a mean follow-up period of 40 months, they found that increasing FPG levels at 12 months post-transplantation were associated with an increasing incidence of adverse CV events (118). However, they found no association between FPG levels and mortality. The short follow-up period may be one of the reasons for a lack of this association. With longer follow-up in our study, we have demonstrated that a higher FPG level at 12 months is associated with long-term mortality. This important observation illustrates the fact that any level of hyperglycaemia associates with an increased the risk of mortality. However, being an observational study, it is not possible to say whether managing this hyperglycaemia better would have altered the outcome.

Despite refinements in immunosuppression regimens, NODAT therefore remains a significant problem associated with poor patient outcomes. In this study, steroid use at 12 months, cumulative prednisolone dose, the type of CNI and tacrolimus levels were not associated with mortality in multivariate analysis (Tables 5.11a and 5.11b). If steroid use and tacrolimus levels are taken as surrogates for the intensity of immunosuppression, these results suggest that in KTRs who died, immunosuppression was not directly associated with mortality in survival analysis. In other words, the adverse effect of NODAT was independent of the type and intensity of immunosuppression. However, we did not have data on the causes of death, and hence it is not possible to demonstrate whether or not the increased mortality associated with NODAT was due to cardiovascular causes alone or due to malignancy and infections. Interestingly, evidence is emerging in the general population that in addition to vascular disease, diabetes is associated with premature death from cancers and infectious diseases, independent of several major risk factors (219). Nevertheless, since there

was clustering of the traditional CV risk factors in patients with NODAT (Table 5.12), we speculate that these factors contributed towards CV morbidity and mortality, leading to worse patient outcomes. In fact, systolic BP, S. creatinineand BMI were independently associated with mortality in multivariate survival analysis (Table 5.11).

Our study has certain limitations apart from the inherent limitations of a retrospective study. There were no data on the family history of T2DM. FPG, but not OGTT, was used to diagnose diabetes both before and after transplantation. Therefore, the burden of T2DM may have been underestimated pre-transplantation leading to KTRs with T2DM being included in the study. Similarly, the incidence of NODAT may also have been underestimated by not using OGTT (181). Since we did not have HbA1c data, it was not possible to examine the effect of the level of hyperglycaemic control on outcomes. Finally, the majority of the patients in our study were caucasian and hence the results may not be valid in a non-caucasian population.

In conclusion, newer immunosuppression protocols using induction therapy and low-dose tacrolimus appear to confer a lower risk of developing NODAT (half the odds) compared with older protocols with high-dose tacrolimus and no induction therapy. We have demonstrated that in the CTU, the actual delivered doses of tacrolimus and steroid have progressively decreased with time, and this almost certainly contributes to reduced β-cell toxicity and insulin resistance respectively. NODAT continues to exert a significant adverse effect on patient outcomes despite refinements in immunosuppression protocols. Patients with NODAT accumulated other CV risk factors too. Managing these risk factors in conjunction with managing the hyperglycaemia should be part of the strategy to improve long-term outcomes in KTRs. Further approaches to minimise the development of post-

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transcript hyperglycaemia and NODAT need to be examined. However, the effect these approaches may ultimately have on long-term patient survival will be also need to be studied closely.

CHAPTER 6: CONCLUSIONS AND FUTURE WORK

Despite improvements in immunosuppression regimens and acute rejection rates in recent years, death with a functioning graft remains an important cause of graft loss after kidney transplantation. Adverse CV events are a leading cause of mortality in KTRs. Other studies have shown that post-transplant hyperglycaemia and NODAT adversely impact patient outcomes by causing an excess of CV events (117, 118, 183). With increasingly older ESRF patients being accepted for kidney transplantation, together with the epidemic of obesity in the general population, there is concern that NODAT will pose significant challenges in the coming years. Therefore, a better understanding of the causes, associations, prediction and outcomes of NODAT in the modern era of kidney transplantation is essential.

To summarise what is already known on these issues: 1. Gold-standard measures of insulin sensitivity and secretion have been used to elucidate the pathophysiology of NODAT, but these techniques are invasive and time-consuming. Although the fasting insulin indices of HOMA and McAuley's index have been validated in KTRs, the utility of these indices in predicting NODAT is unknown; 2. The OGTT is established as a sensitive tool to detect NODAT and glucose intolerance in KTRs. It has been used to study glucose metabolism early after transplantation, but the progression of glucose intolerance diagnosed late after transplantation is not well defined. Also, there are no studies linking the metabolic syndrome (MS) diagnosed >12 months after transplantation and its effect on subsequent glucose intolerance; 3. The reported incidence of NODAT varies widely, presumably due to varying definitions, differences in immunosuppression regimens and different post-transplant periods studied. Longitudinal studies have nevertheless reported an increasing incidence of NODAT up to ten years after transplantation. Immunosuppression drugs are thought to play an

important role in the causation of post-transplant hyperglycaemia and NODAT. In more recent years, significant changes have occurred in immunosuppression regimens. The incidence of NODAT in the context of these newer regimens is not clearly known; 4. Finally, NODAT has been shown to increase mortality in observational studies. However, the effect of NODAT diagnosed early after transplantation using fasting plasma glucose has not been examined thoroughly. Moreover, the question remains as to whether the effect of NODAT on mortality is related to other complications of immunosuppression (malignancy and infection) or traditional CV risk factors.

Key data and findings from the three studies presented in this thesis are shown in Table 6.1. In the Cic vs. Tac study, we analysed non-diabetic KTRs selected from an RCT with a follow-up period of more than ten years. We calculated fasting insulin indices to describe the pathophysiology of NODAT and predict future NODAT. In this study, we have shown that there is increasing insulin resistance (IR) and a lack of compensatory increase in insulin secretion in patients developing NODAT. There was an early increase in IR in those treated for rejection and a late increase in those with no rejection, suggesting the effect of increased steroid use in those treated for rejection. Moreover, patients with NODAT had higher IR and lower insulin secretion than those without NODAT. Increasing age and exposure to tacrolimus were risk factors for developing NODAT, and we know that tacrolimus has an adverse effect on β-cell function. These findings have some similarities to the currently described pathophysiology of T2DM – inadequate β-cell function in the face of chronic over-nutrition and insulin resistance (19).

Table 6.1 – A summary of key data and findings from the three studies presented in this thesis

Study name	Design and N	Diagnostic criteria for DM and incidence / prevalence of NODAT	Risk factors for NODAT	Risk factors for mortality	Other key findings
Cic vs. Tac	Randomised trial follow-up and sub-group analysis (N=118)	FPG≥7.0 mmol/l or medication use; 1-year incidence 37%, 10-year incidence 42%	Age, exposure to tacrolimus, FPG>5.6 mmol/l	Age, NODAT in 3 months, S. creatinine at 12 months	Insulin resistance higher in NODAT patients than in non-NODAT patients; no compensatory increase in insulin secretion in NODAT patients; insulin indices did not predict NODAT
OGTT follow-up	Longitudinal observational (N=76)	OGTT WHO criteria; NODAT prevalence increased from 13% to 24% over six years	FPG and abnormal OGTT at baseline	Not studied	Increase in BMI and WHR, decrease in CNI levels and steroid use with time; prevalence of abnormal glycaemia increased from 42% to 61% over six years; MS associated with progression of glucose intolerance
Historic vs. recent transplantation cohort	Cohort observational study (N=616)	FPG≥7.0 mmol/l or medication use; 1-year incidence (old era 19%, new era 12%); 5-year incidence (old era 26%, new era 17%)	Old era, age, BMI, male gender	NODAT in 12 months, FPG at 12 months, S. creatinine, systolic BP	Dose and blood levels of tacrolimus lower in the new era; tacrolimus levels not associated with NODAT or mortality on multivariate analysis; NODAT patients accumulated other CV risk factors

Also in the Cic. vs. Tac study, insulin indices calculated pre-transplantation or early post-transplantation using HOMA and the McAuley's index were not associated with the development of NODAT, whereas traditional risk factors for DM such as increasing age and higher FPG were found to be important. In fact, pre-transplantation HOMA-IR and McAuley's index were not significantly different between patients who did, and those who did not develop NODAT. Exposure to tacrolimus and FPG >5.6 mmol/l at three months post-transplantation were associated with a higher risk of developing NODAT during follow-up. Pre-transplantation levels of FPG, S. triglycerides and BMI were similar between the future NODAT and non-NODAT groups, and these factors of MS did not predict NODAT.

In the OGTT follow-up study, we assessed KTRs with an FPG >5.6 mmol/l at baseline, who then underwent paired OGTTs. Within this group, we showed that there is progression of glycaemic abnormalities even at a late stage after transplantation. Over a period of six years, the prevalence of NODAT increased from 13% at baseline to 24% at follow-up while the prevalence of an abnormal OGTT result increased from 42% to 61%. This occurred in the context of lower CNI levels (both tacrolimus and ciclosporin) and less prevalent steroid use at follow-up. The presence of MS was associated with this progression of glucose intolerance.

Fifty-seven percent of patients who had MS at baseline progressed to a higher degree of glucose intolerance compared with 27% of those without MS. The only MS factor which was significantly associated with this progression on multivariate analysis was an FPG level >5.6 mmol/l. Factors associated with the development of NODAT were baseline 0-hr glucose and an abnormal baseline OGTT result. The type of CNI, CNI blood levels and steroid treatment were not associated with NODAT development or progression of glucose intolerance at this late period after transplantation.

Based on these findings from the OGTT study, we suggest that all KTRs with FPG between 5.6-6.9 mmol/L and those who meet the criteria for MS irrespective of FPG should undergo an OGTT for further risk stratification. This strategy can be used to identify patients at risk of progression so that lifestyle modification interventions can be introduced early to gain maximal benefits.

As described in Chapter 2 and demonstrated in Chapter 5, immunosuppression regimens for kidney transplantation have evolved considerably over the last two decades. In Cardiff, there is now extensive use of induction immunosuppression therapy and MMF use is near universal. Tacrolimus has clearly become the CNI of choice: 96% of KTRs receiving tacrolimus in the new era (2004-2009) compared to 53% in the old era (1997-2001). Furthermore, target blood concentrations of tacrolimus as well as actual achieved levels have decreased with time. Biopsy proven AR rate has decreased from 37% in the old era to 27% in the new era. Cumulative exposure to prednisolone was also lower in the new era.

In the historic vs. recent cohort study, we have demonstrated that the incidence of NODAT in Cardiff is decreasing. The 12-month incidence fell from 19% in the old era to 12% in the new era. The cumulative incidence over five years also fell from 26% to 17%, although this decrease was largely due to a difference in the 12-month incidence. On multivariate analysis, being transplanted in the old era, older age and higher BMI were associated with the development of NODAT. However, when "era" was replaced by CNI levels and steroid exposure, these factors were not associated with NODAT development, suggesting that other factors that were different between the eras may also be important.

Interestingly, KTRs who developed NODAT in the new era had a higher BMI than those with NODAT in the old era. In keeping with the overall trend, new era NODAT patients had lower

tacrolimus levels than the old era NODAT patients. These findings support the notion that similar to T2DM, NODAT develops due to unmasking of pre-existing risks for DM. In other words, older and obese patients are already at risk for developing diabetes, and further stress on the β-cells from CNIs unmasks overt hyperglycaemia. In the new era, the intensity of β-cell toxicity needed to cause NODAT may have been lower compared to that needed in the old era, given the higher BMI of NODAT patients in the new era contributing to lower insulin sensitivity. The higher blood tacrolimus levels in the old era presumably resulted in a higher intensity of β-cell toxicity, thus unmasking NODAT in susceptible KTRs with even lower BMIs. We know from other studies that in KTRs, there is a dose-dependent effect of tacrolimus levels on insulin secretion (216). Despite these observations, in multivariate analysis corrected for age, gender, BMI and era, tacrolimus levels at 6 or 12 months, and cumulative prednisolone dose in the 1st year were not associated with an increased risk of NODAT.

In the context of a post-transplant state, correction of uraemia causes a reversal of poor appetite and the catabolic state; this can consequently lead to weight gain post-transplantation. Not surprisingly, in all three studies, there was an increase in BMI of KTRs post-transplantation. This was seen in the first year post-transplant (Cic vs. Tac and incidence studies) as well as late post-transplant (OGTT follow-up study).

In the Cic vs. Tac follow-up study, we showed that the early development of NODAT within three months was associated with increased all-cause mortality. There was no association between markers of increased immunosuppression (treatment for AR, steroid use) and mortality. Survival curves differed between NODAT and non-NODAT KTRs from only four years after transplantation. Since the vascular complications of diabetes take much longer to

develop, it is likely that KTRs who developed NODAT had pre-existing IFG or IGT which was unmasked post-transplantation. Indeed, 33% of patients who developed NODAT during follow-up had IFG pre-transplantation, compared to 15% of those without NODAT. IFG and IGT are themselves known to be risk factors for cardiovascular disease (120). Therefore, it is plausible that the cardiovascular morbidity these patients endured due to IFG / IGT during the uraemic state was accelerated by the adverse metabolic milieu brought on by overt post-transplant hyperglycaemia / NODAT.

In the historic vs. recent cohort study also, all-cause mortality was higher in patients with NODAT compared to those without NODAT. Also, fasting plasma glucose by itself as a continuous variable was associated with mortality. This result was independent of the type of CNI, tacrolimus levels, cumulative prednisolone dose and the era of transplantation. These results confirm the findings from previous cohort studies from the 1990s and show a similar adverse effect of hyperglycaemia and NODAT on patient survival even in the current immunosuppression era. Again, the decrease in patient survival on K-M analysis started to become apparent from approximately 3 to 4 years after transplantation. As discussed above, this duration of the presence of diabetes is too short for hyperglycaemia alone to cause cardiovascular complications and consequent mortality. However, we have shown that KTRs with NODAT also had worse BP, BMI and S. triglyceride levels which increase CV risk. In fact, on multivariate analysis, a higher systolic BP and higher S. creatinine at 12 months were associated with increased mortality. The type of CNI, cumulative steroid use and tacrolimus levels at 6 or 12 months were not associated with mortality. Taken together, these results suggest that the adverse effect of hyperglycaemia on patient survival is independent of the level of immunosuppression, and is related to other traditional CV risk factors.

Our central hypothesis was that NODAT is distinct from T2DM and is due to factors unique to the transplant setting, of which the predominant factor is the use of CNIs and steroids early after transplantation, and that traditional risk factors for T2DM are the more significant factors late after transplantation. Results from the studies described in this thesis support this hypothesis. Specifically, tacrolimus was found to increase the risk of NODAT but did not increase the risk of mortality. Based on these results and building from evidence from the literature, we propose a model that links the risk factors for post-transplant hyperglycaemia and NODAT to their association with increased mortality (Figures 6.1 and 6.2) -

A. Traditional risk factors for T2DM such as increasing age, BMI and higher plasma glucose levels are also risk factors for developing NODAT both early and late after transplantation. MS is associated with the progression of glucose intolerance in the late post-transplant period.

B. Tacrolimus is more likely to cause NODAT than ciclosporin. The effect of tacrolimus on hyperglycaemia is predominantly seen early after transplantation with higher blood levels of the drug.

C. Although the type of CNI has an effect on the incidence of NODAT, the type of CNI has no effect on either short term or long-term mortality.

D. High plasma glucose levels and a diagnosis of NODAT in the first year are associated with worse all-cause mortality. There is also clustering of the traditional CV risk factors of high BP, lipid levels and BMI in KTRs with NODAT, which contributes to the adverse outcomes. Aggressive management of all these MS variables is essential

to reduce CV morbidity and mortality, rather than managing the hyperglycaemia in isolation.

Figure 6.1 – Model depicting pre- and post-transplant incidence curves for DM
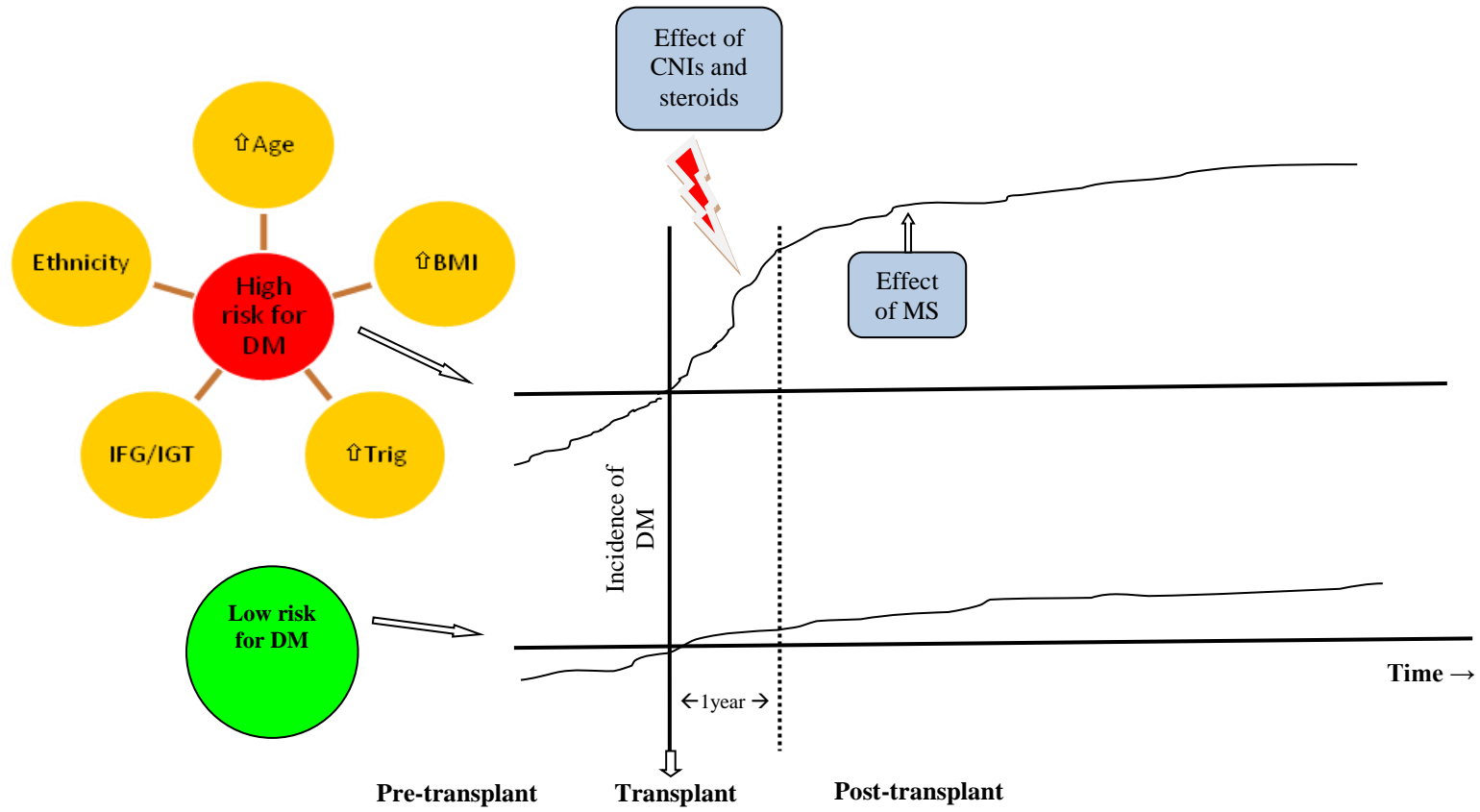
Figure 6.2 – Model linking cardiovascular risk factors and NODAT with post-transplant mortality

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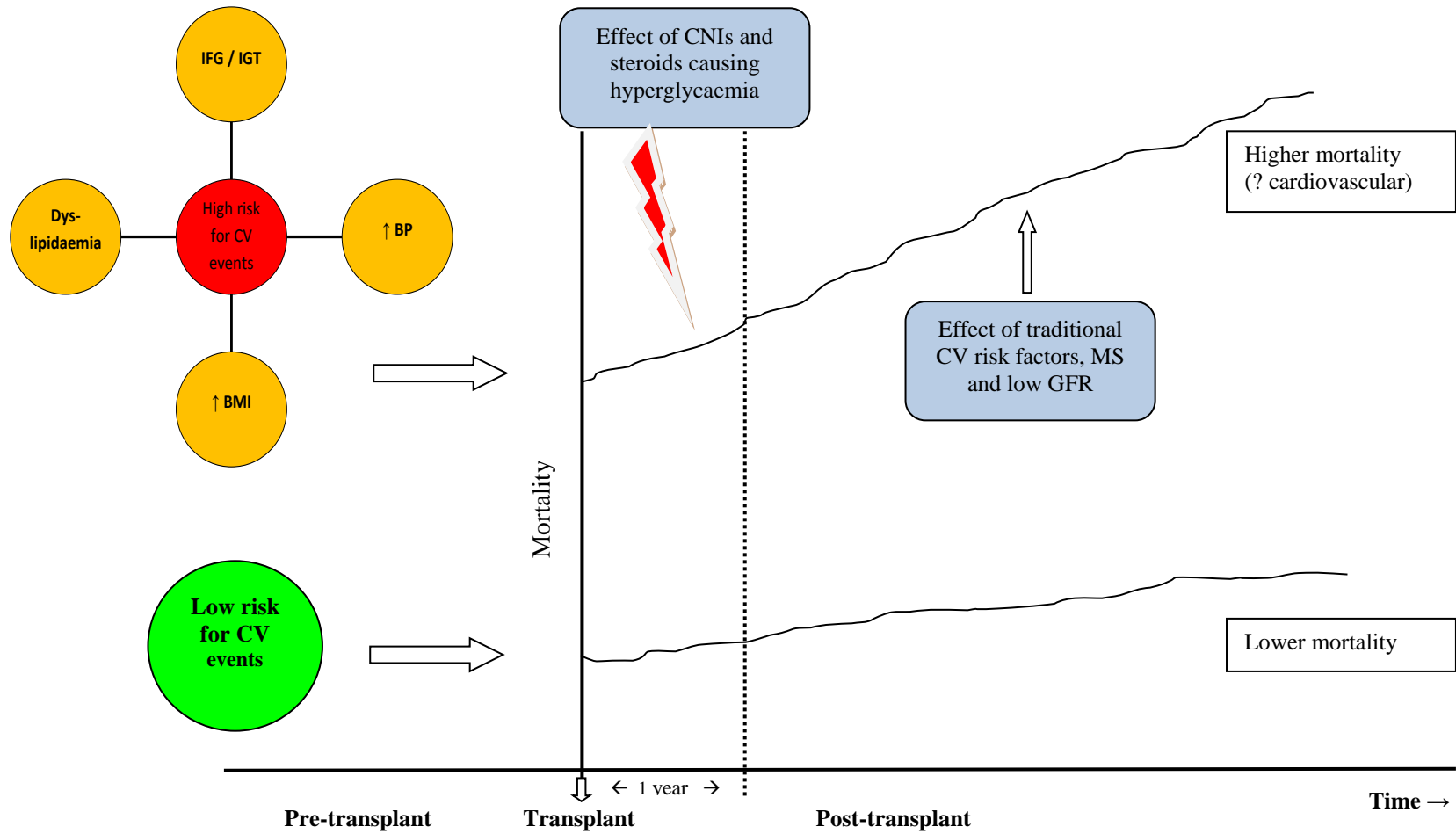
There is a lack of long-term efficacy and safety studies in KTRs on the use of hypoglycaemic drugs, especially the newer agents. The results of the RCTs that are currently underway to study the pharmacological management of NODAT will be crucial. However, it is predicted that although glucose-lowering and NODAT-preventing interventions will reduce the incidence of NODAT and reduce HbA1c, they may not have a beneficial effect on mortality if other CV factors are also not managed well at the same time. In fact in patients with T2DM, a recent meta-analysis showed that there were no benefits of intensive glucose lowering on all-cause mortality and cardiovascular deaths (220).

Adjusting immunosuppression further by reducing exposure to tacrolimus might reduce the incidence of NODAT, but will not reduce the mortality from NODAT, which is due to an effect of MS variables and not due to elevated blood glucose alone. Future studies should therefore focus on lifestyle modification strategies and their impact on metabolic and cardiovascular abnormalities in a prospective manner in both pre- and post-transplant patients. Lifestyle interventions can help in better management of body weight, blood glucose, blood lipids and blood pressure, all of which need to be controlled simultaneously in order to improve outcomes.

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