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Hyperglycaemia before and after renal transplantation

Faculty of Medicine
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Oslo, January 2010
Henrik Andreas Bergrem
### 3. ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>11β-HSD1</td>
<td>11-β-hydroxysteroid dehydrogenase type 1</td>
</tr>
<tr>
<td>2h-PG</td>
<td>2-hour post-challenge plasma glucose</td>
</tr>
<tr>
<td>A1C</td>
<td>Haemoglobin A1C</td>
</tr>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CNI</td>
<td>Calcineurin inhibitor</td>
</tr>
<tr>
<td>CsA</td>
<td>Cyclosporine A</td>
</tr>
<tr>
<td>DBN</td>
<td>Diabetic nephropathy</td>
</tr>
<tr>
<td>DM</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>ESRD</td>
<td>End stage renal disease</td>
</tr>
<tr>
<td>FPG</td>
<td>Fasting plasma glucose</td>
</tr>
<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid receptor</td>
</tr>
<tr>
<td>GRE</td>
<td>Glucocorticoid responsive element</td>
</tr>
<tr>
<td>HGO</td>
<td>Hepatic glucose output</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired fasting glucose</td>
</tr>
<tr>
<td>IGT</td>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>IR</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td>ISec</td>
<td>Insulin secretion</td>
</tr>
<tr>
<td>MAR</td>
<td>Missing at random</td>
</tr>
<tr>
<td>MCAR</td>
<td>Missing completely at random</td>
</tr>
<tr>
<td>MNAR</td>
<td>Missing not at random</td>
</tr>
<tr>
<td>NODAT</td>
<td>New onset diabetes mellitus after transplantation</td>
</tr>
<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
</tr>
<tr>
<td>PHYGG</td>
<td>Posttransplant hyperglycaemia</td>
</tr>
<tr>
<td>PTH</td>
<td>Parathyroid hormone</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic</td>
</tr>
<tr>
<td>RTx</td>
<td>Renal transplantation</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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4. LIST OF PAPERS


5. INTRODUCTION

Renal transplantation (RTx) is the best treatment option for end stage renal disease (ESRD) (1). In the early days of clinical transplantation, early graft rejection was the most important clinical problem after RTx. Modern immunosuppressive drugs have significantly reduced the magnitude of this problem, and shifted the focus towards improving the long term patient and graft outcomes. Increasing efforts are thus being made to lower the burden of cardiovascular disease, which is a major cause of death in RTx patients (2;3). The increased cardiovascular risk in RTx patients seems attributable to traditional risk factors such as old age and male gender, but also to abnormalities in glucose regulation occurring after RTx (3;4).

Diabetes mellitus (DM) and other abnormalities in glucose regulation are common after RTx. This is often to be expected, since patients with diabetic nephropathy (DBN) or other long-standing DM before RTx will normally continue to have DM after surgery. However, even in non-diabetic populations, ESRD predisposes to pretransplant glucose intolerance by being an insulin resistant state (5). This predisposition often becomes apparent after RTx, when the incidence of DM increases (6). Some 10-20% of the patients without pretransplant DM develop new onset DM after RTx (NODAT), and 20-40% have milder forms of hyperglycaemia (7).

5.1. Epidemiology of hyperglycaemia

The global burden of hyperglycaemia has reached epidemic proportions. As defined by the World Health Organization (WHO) criteria for hyperglycaemia, it is estimated that 285 million people have DM worldwide (6.4%), and that 438 million will have the disease by 2030 (8). Type 2 DM is estimated to account for more than 90% of all cases of DM. Only some 50% of all prevalent cases of DM have been diagnosed, with the highest proportion diagnosed in North America (70%) and the lowest in Africa (30%). In addition to DM, 344 million people are estimated to have impaired glucose tolerance (IGT), corresponding to roughly 8% of the world adult population. Compared to DM and IGT, the prevalence of impaired fasting glucose (IFG) is less well examined. The overall prevalence has been estimated to roughly 5% using the WHO criteria (9;10). As compared to these criteria, the former American Diabetes Association (ADA) criteria were thought to raise the apparent prevalence of IFG by a factor of two to three (9). Accordingly, a 15% prevalence was recently
reported (11). No large studies have been performed, however, subsequent to the recent modification of diagnostic criteria introduced by the ADA as of January 2010 (12).

DM leads to macro- and microvascular complications. The all-cause mortality rate in patients with DM is two to four times higher than in the general population, much due to macrovascular disease, which accounts for roughly 50% of all deaths in these patients (13). Diabetic retinopathy is the leading cause of blindness in adults below 75 years of age in developed countries, and occurs in more than 60% of patients with DM within 15-20 years of diagnosis (14). Peripheral neuropathy affects an estimated 20% of diabetic patients (15). Between 12% and 45% of incident ESRD is due to DM worldwide, making DM the most common cause of ESRD in countries such as the U.S., Japan and Korea (16). The incidence of ESRD due to type 2 DM is reported to increase by 10% annually in some regions (17;18).

5.2. Pathophysiology of hyperglycaemia

The maintenance of normal glucose homeostasis requires a precise regulation of glucose production and disposal. Insulin facilitates the tissue uptake of glucose and suppresses glucose production, and is therefore the key regulator of glucose homeostasis. Defects in insulin action or secretion can result in insufficient disposal of glucose, an excessive production of glucose, or a combination of both factors, leading to hyperglycaemia.

In the fasting state, hyperglycaemia is primarily thought to reflect an elevated glucose production. This can occur if glucose-producing organs are resistant to the anti-gluconeogenic effect of insulin, or if insulin levels are insufficient to suppress the production of glucose. Postprandial hyperglycaemia mainly results from a reduced glucose disposal secondary to varying degrees of insulin resistance (IR) and impaired insulin secretion (ISec) (19;20). The proposed pathogeneses of fasting and postprandial hyperglycaemia are illustrated in Figure 1.

DM is a diverse group of disorders characterized by defects in insulin secretion (type 1 DM) and most often also insulin sensitivity (type 2 DM), leading to overt forms of fasting or postprandial hyperglycaemia, or a combination of both. Type 1 DM results from a progressive destruction of pancreatic β cells, whereas type 2 DM arises in a combined context of IR and impaired ISec. The development of DM is commonly thought to be preceded by a period of intermediate degrees of hyperglycaemia. These are termed IFG or IGT, depending on whether fasting or postprandial hyperglycaemia is clinically predominant. The transition from intermediate hyperglycaemia to DM is often explained by increasing defects in ISec (21). In the development of type 2 DM, IR is often considered the initial defect. In the presence of IR,
Figure 1. Contribution of different glucose compartments in glucose homeostasis.

A. Normal fasting state. Hepatic glucose output (HGO) is the main source of glucose. B. Normal postprandial state. Oral intake is the main source of glucose. Insulin facilitates peripheral and hepatic glucose uptake, and suppresses HGO. C. Fasting hyperglycaemia. HGO is increased due to insulin deficiency or hepatic insulin resistance (IR). In the presence of peripheral IR or insulin deficiency, this results in hyperglycaemia. Peripheral glucose uptake is increased by an insulin independent mass effect to accommodate the increased glucose output (22). D. Postprandial hyperglycaemia. IR or insulin deficiency results in reduced peripheral and hepatic insulin mediated glucose uptake, as well as in sustained HGO, leading to hyperglycaemia. Oral intake is the dominant source of glucose, although HGO also contributes. The glucose mass effect ensures a maintained peripheral glucose uptake despite the insulin resistance or deficiency (23). [Schematic figure created by HA Bergrem. The magnitude of the contribution from each compartment is subject to debate; arrow thicknesses should be interpreted accordingly. Glucagon and other substances are omitted for clarity.]
overt DM is avoidable through a compensatory hypersecretion of insulin in the early phases of the disease. Once β cell function deteriorates, DM becomes clinically manifest.

5.3. Hyperglycaemia in end stage renal disease

5.3.1. Epidemiology

The prevalence of undiagnosed hyperglycaemia (DM or intermediate hyperglycaemia) among ESRD patients has not been studied in large populations. Small studies have indicated a prevalence of undiagnosed hyperglycaemia in the range of 30-50% as determined by the oral glucose tolerance test (OGTT) (24;25). The prevalence of acknowledged DM was 33% among all patients entering renal replacement in Norway in 2008 (26). In the majority of these patients (55%), DM was listed as the primary cause of ESRD. Although similar figures have been reported in other countries (18), the overall prevalence of acknowledged DM in ESRD (i.e. DM with or without DBN) is likely to display significant geographical variability.

Due to the lack of studies examining intermediate hyperglycaemia and undiagnosed DM, the prognostic implication of these conditions in ESRD is largely unknown. Increased 1-year mortality rates have been described in hemodialysis patients with IFG as compared to otherwise comparable patients having normal glucose levels (27). As for patients with acknowledged DM at the time of receiving the ESRD diagnosis, a significant excess mortality risk is observed as compared to patients with ESRD and no DM (18). Macrovascular disease accounts for nearly half of all deaths in diabetic ESRD patients (17). RTx confers a significant survival benefit in ESRD patients with type 1, and probably also type 2 DM (28).

5.3.2. Pathophysiology

Reduced renal function is associated with abnormal glucose metabolism even in the absence of DM (29). IR and increments in postprandial plasma glucose are inversely related to glomerular filtration rate (GFR) in subjects with normal glucose tolerance and mild to moderate chronic kidney disease (CKD) (30). The presence of IR is more pronounced among patients having GFR levels below 50 mL/min/1.73m² (31), and is nearly universal in ESRD (5). Nonetheless, IR is often compensated by increments in ISec, and also by the reduction in insulin clearance that accompanies renal failure in advanced CKD. This leads to elevated fasting insulin levels that are capable of controlling the degree of fasting hyperglycaemia (32).
Even though IR and renal dysfunction are related, both the nature and direction of this relationship are unclear. The presence of IR may damage the kidneys through pathways involving inflammation, either directly or via mechanisms related to other cardiovascular risk factors, such as old age, obesity, hypertension or dyslipidaemia (30;33). It is well known, however, that the insulin sensitivity in ESRD patients improves following hemodialysis, and that the improved glucose tolerance remains improved for up to one week after cessation of hemodialysis (34). This strongly suggests that IR in uraemic patients is related to factors that are specific to renal failure. Among several possible risk factors (Table 1), nitrogenous compound retention is at present the dominant hypothesis; it supports the role of a dialysable solute, and the insulin-sensitizing effects of low-protein diets in uraemia (35).

Table 1. Risk factors for insulin resistance in uraemia

<table>
<thead>
<tr>
<th>General</th>
<th>Uraemia related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Nitrogenous compound retention (36;37)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Protein energy wasting (38)</td>
</tr>
<tr>
<td>Obesity</td>
<td>Vitamin D deficiency (39)</td>
</tr>
<tr>
<td>Family history</td>
<td>Metabolic acidosis (40)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Anaemia (41)</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td>Calcium-phosphate derangements (42;43)</td>
</tr>
<tr>
<td></td>
<td>Secondary hyperparathyroidism (44)</td>
</tr>
</tbody>
</table>

Abnormalities directly affecting ISec are less consistently present as compared to the defects in insulin sensitivity and clearance in ESRD (45;46). Nonetheless, as in type 2 DM, β cell defects are often involved in cases of overt hyperglycaemia (47). The pathogenesis of these defects is nonetheless poorly understood. While some studies have suggested that hyperparathyroidism or hypocalcaemia could impair ISec (42;46;48;49), other studies have disputed these findings (44;50-53). The altered metabolism of incretins in CKD could play a role (54). It is also likely that reduced ISec in ESRD is related to old age, genetic factors and toxic effects of hyperglycaemia, free fatty acids or other substances (21), as it is in type 2 DM.
5.4. Hyperglycaemia after renal transplantation

5.4.1. Epidemiology

The incidence of DM after RTx is higher than before RTx (6). Accordingly, a considerable proportion of patients develops NODAT. Although reported incidences vary widely (55), it may be assumed that some 10-20% of the patients develop NODAT, and that 20-40% have intermediate hyperglycaemia (7). The incidence of NODAT is highest during the initial few months after RTx, and declines gradually thereafter (6;56-59).

NODAT is associated with increased mortality (57;60-62), primarily through macrovascular disease, which accounts for 30-50% of all deaths in RTx patients (3;16;63). NODAT may also increase the risk of graft loss (57;61) and non-fatal macrovascular events (61;63;64). The spectrum of diabetic complications due to NODAT is otherwise similar to that observed for type 2 DM, but complications appear to occur at a higher rate (65). As in the general population, even non-diabetic levels of hyperglycaemia have been associated with increased mortality and morbidity in RTx patients (4;66).

5.4.2. Pathophysiology

Even though RTx helps restore renal function, patients continue to display IR after RTx (67). This creates a sustained demand for compensatory elevations in ISec, which is further augmented by the increased renal clearance of insulin that accompanies restoration of renal function. The maintenance of normoglycaemia after RTx therefore places high demands on β-cell function. Accordingly, an insufficient residual β-cell capacity is a key component in the development of overt posttransplant hyperglycaemia (PHYG) (68;69).

As illustrated in Table 2, PHYG is associated with both pre-existing and RTx related risk factors (7;55;70-72). Among these factors, immunosuppressive therapy arguably constitutes the single most important factor (55). Prednisolone and calcineurin inhibitors (CNIs) predispose to NODAT, and probably also intermediate hyperglycaemia (59;73-75). Inhibition of ISec appears to be the main mechanism by which CNIs exert their diabetogenic effect (76;77). Prednisolone may also inhibit ISec to some extent, but primarily induces IR in skeletal muscle and liver, leading to impaired glucose disposal and increased glucose production (78). Within the group of pre-existing factors, pretransplant glucose levels provide information that may not be fully accounted for by traditional risk factors (79-82).
Due to the restoration of renal function, it may not be expected that hyperglycaemia should correlate with similar risk factors after RTx as in ESRD. Nonetheless, renal function is not normal after RTx, and neither is the metabolism of substances such as calcium, phosphate, parathyroid hormone (PTH), haemoglobin or nitrogenous compounds (collectively termed ‘CKD related factors’ in Table 2). It may therefore be premature to conclude that these substances only relate to hyperglycaemia in ESRD. Surprisingly, the role of these substances has not previously been addressed in relation to PHYG.

**Table 2. Risk factors for posttransplant hyperglycaemia**

<table>
<thead>
<tr>
<th>Pre-existing</th>
<th>Transplant related</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>CNI</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Steroids</td>
</tr>
<tr>
<td>HCV</td>
<td>Acute rejection¹</td>
</tr>
<tr>
<td>Family history¹</td>
<td>Increased insulin clearance¹</td>
</tr>
<tr>
<td>Obesity¹</td>
<td>HLA mismatch¹</td>
</tr>
<tr>
<td>Pretransplant glycaemia¹</td>
<td>Donor vital status¹</td>
</tr>
<tr>
<td>CMV¹</td>
<td>CKD related factors¹,²</td>
</tr>
</tbody>
</table>

¹These factors have been reported, but are still under debate.
²Commonly encountered abnormalities related to renal function, such as altered metabolism of calcium, phosphate, PTH, haemoglobin, urea/nitrogenous compounds and other substances

5.4.3. **Role of prednisolone**

The risk of PHYG depends on the prednisolone dose, and can be lowered by prednisolone dose reductions (74;83). Low-dose steroid or steroid free immunosuppressive regimens have thus become increasingly common. Nonetheless, these strategies may increase the risk of acute rejection (71). It is also unlikely that dose reductions can eliminate the empirical inter-subject variability in the occurrence of prednisolone effects and side effects, which is even observed between subjects receiving the same weight adjusted doses of prednisolone. This suggests that better tools are needed to determine the optimal prednisolone dosage in individual patients. In RTx recipients, this task is complicated by the possibility that the metabolism of prednisolone may interact with that of other immunosuppressive drugs (84).
The inter-subject variability of prednisolone effects and side effects may partly be attributable to its unusual pharmacokinetics. The protein binding of prednisolone decreases non-linearly from 95% to 60%–70% as the total concentration increases from 200 ng/mL to 800 ng/mL. At low concentrations (≤200 ng/mL), prednisolone binds mainly to plasma transcortin, while at higher concentrations, it increasingly binds to albumin (85). The non-linear protein binding coincides with the observation that certain parameters in prednisolone pharmacokinetics, such as the clearance of unbound drug, have been found dose independent (86). This is likely to have clinical relevance, since biological effects of prednisolone are believed to be exerted by the unbound fraction of the drug (87). Low albumin levels are associated with reduced protein binding and increased glucocorticoid side effects in systemic lupus erythematosus (88), celiac (89) and inflammatory bowel disease (90). The concentration of unbound prednisolone is increased in RTx recipients, due to reduced renal or hepatic clearance (91). Similar findings have been made in liver and lung transplant recipients (92).

The potentially variable combined effects of non-linear protein binding and renal or hepatic impairment create a rationale for prednisolone concentration monitoring after RTx. However, the research effort relating to such monitoring has been very small for prednisolone as compared to more modern immunosuppressive agents (93). Nonetheless, prednisolone meets several of the requirements proposed for therapeutic drug monitoring (87). First, the time from dosing until the appearance of side effects is often long. Second, the therapeutic index of prednisolone is sufficiently narrow to warrant monitoring. Third, the requirement for prednisolone is usually prolonged in RTx recipients. Fourth, reliable methods for measuring prednisolone are available. Finally, a direct association between prednisolone concentration and effects can be demonstrated for several outcomes. Such a relationship has not, however, been demonstrated between prednisolone and posttransplant glycaemia.
6. AIMS OF THE STUDIES

An optimal handling of posttransplant glucose intolerance requires a broad understanding of glucose abnormalities occurring both before and after RTx. The primary goals of the present work were to shed new light on the natural history of glucose tolerance before and after RTx, and to examine some risk factors for the development of posttransplant hyperglycaemia.

6.1. Paper 1

No epidemiological studies have formally assessed both fasting and postprandial glucose to identify patients with hyperglycaemia before RTx. We aimed to determine the prevalence of undiagnosed DM and intermediate hyperglycaemia in renal transplant candidates, and also to study how patients with undiagnosed DM may most effectively be identified.

6.2. Paper 2

The presence of subtle glucose abnormalities before RTx has been found to translate into an increased risk of PHYG. We wanted to verify this finding in a large population, and also address whether the predictive effect of pretransplant glycaemia could be different for fasting and postprandial glucose. In addition, we aimed to examine whether hyperglycaemia may have common metabolic denominators in uraemic and transplanted populations.

6.3. Paper 3

Although the diabetogenic effect of prednisolone after RTx is dose related, there is great variability in the occurrence of glucose intolerance between subjects receiving the same prednisolone dose. We wanted to investigate whether the effect of prednisolone on glucose tolerance may be more precisely evaluated through pharmacokinetic rather than strictly dose related parameters. Specifically, we studied whether the exposure to unbound prednisolone, the biologically active component of prednisolone, is associated with glucose tolerance.
7. PATIENTS AND METHODS

7.1. Study population and design

The present work contains three articles investigating glucose tolerance in relation to RTx. In these papers, glycaemia is assessed before RTx (Paper 1), before and short term after RTx (Paper 2), and long term after RTx (Paper 3).

7.1.1. Paper 1

This is a cross-sectional population based study of glucose metabolism at the time of referral for RTx in non-diabetic RTx candidates in Norway. All adult patients with non-diabetic ESRD referred from renal units in Norway to Rikshospitalet University Hospital for a 1st single RTx between September 2002 and February 2009 were eligible (n=1111). Patients with known DM prior to referral were excluded (n=77), along with patients having incomplete pretransplant glucose data (n=145). A total of 889 patients were therefore included. Patients and their data were identified using the hospital records of Rikshospitalet University Hospital. All patients provided a written informed consent for the use of their data, and the project was performed in accordance with the Declaration of Helsinki.

7.1.2. Paper 2

This is a retrospective cohort study of risk factors for PHYG early after RTx. Specifically, the role of pretransplant glycaemia and correlates of posttransplant renal function were examined. The incidence of NODAT was recently addressed in a report on patients who underwent RTx in Norway between October 2003 and October 2005 (94). Extended data from this cohort were made available for our analysis. Among all eligible patients (n=500), we included all adults who attended the posttransplant follow-up and were without overt pretransplant DM (n=301). Overt pretransplant DM was defined as having known DM before the RTx work-up (with or without DBN), alternatively as the initiation of glucose lowering drug therapy between work-up and RTx. The study cohort contained a subset of patients who were studied in Paper 1 (n=257), in addition to 44 patients receiving a re-transplant. Patients and their data were identified using the hospital records of Rikshospitalet University Hospital. All patients
provided a written informed consent for the use of their data. The project was approved by the local Data Inspectorate and performed in accordance with the Declaration of Helsinki.

7.1.3. **Paper 3**

This is a cross-sectional study of the association between prednisolone pharmacokinetics and glucose tolerance in stable RTx recipients. Patients were recruited from the outpatient clinic at Rikshospitalet in 1980-81, and included all adult outpatients having non-diabetic renal disease, no current DM, a stable graft function and ≥6 months of follow-up (n=187). Patients were examined at a median of roughly 5 years posttransplant, and constituted some 50% of all non-diabetic RTx recipients alive in Norway at the time. Based on these data, a report was published in 1985 on the pharmacokinetics of prednisolone in this population (95). A number of patients also agreed to perform an OGTT during the study (n=131). These data were not given priority in the report published in 1985, but have now been re-analyzed to include the unpublished OGTT. After excluding subjects taking prednisolone twice daily (n=21) or having an extreme 2-hour post-challenge plasma glucose (2h-PG; >19 mmol/L, n=2), 108 patients (41 females, 67 males) were included. All used prednisolone, in combination with either azathioprine (n=99) or cyclophosphamide (n=9). Participation was voluntary and based on informed consent. The present reanalysis of historical data was approved by the Southern Norway Regional Committee for Medical Research Ethics.

7.2. **Glucose measurements**

7.2.1. **Paper 1**

Along with a résumé of each patient’s medical history, the referral for RTx in Norway contains a standardized work-up form reporting the result of a pretransplant standard 75g OGTT that is mandatory in all patients without acknowledged DM. The OGTT is analyzed according to local hospital standards and should include a specification of the test material used (plasma vs. whole blood). Unfortunately, a specification of the test material used was lacking in some 90% of patients. We therefore asked referring centres to provide written information on the material used within specific time periods. All centres were subsequently contacted per phone to confirm the information provided. The information was used to specify a test material for each patient lacking such data. In 81 patients, the OGTT result was
reported in venous whole blood rather than plasma. These results were converted to plasma equivalent values through multiplication by a factor of 1.11 (96).

7.2.2 Paper 2

Pretransplant OGTT data were retrieved as described for Paper 1. Whole blood results were only reported in two patients, and results were considered plasma equivalent.

At follow-up 10 weeks posttransplant, all patients underwent a repeat OGTT at our centre, except if DM had been diagnosed between RTx and follow-up. This OGTT was analyzed in venous whole blood by a modified glucose dehydrogenase method. Results were classified according to WHO whole blood criteria for hyperglycaemia (see below) (97), but reported as plasma equivalent in the published article (96).

7.2.3. Paper 3

Participants were admitted for a 3-day inpatient data collection. On the morning of day 2, prednisolone analyses were performed as described below. On the morning of day 3, a standard 75g OGTT was performed with venous plasma glucose measured at 30-minute intervals using an enzymatic colorimetric glucose oxidase method. Glucose tolerance was assessed using 2h-PG or glucose area-under-the-curve (AUC) calculated by the trapezoid rule.

7.3. Classification of glucose tolerance

The classification of patients into categories of glycemia is intended to delineate transitions in the risk for complications of hyperglycaemia and progression to overt DM. The WHO and ADA diagnostic criteria differ with respect to IFG and normal fasting glycaemia, and as of year 2010 even with respect to the use of haemoglobin A1C (A1C) for diagnosing DM. The WHO criteria were used in Paper 3, and also for the pretransplant values in Paper 2 and the current summary of all three papers [DM: fasting plasma glucose (FPG) ≥7.0 mmol/L or 2h-PG ≥11.1 mmol/L; IGT: FPG <7.0 mmol/L and 2h-PG 7.8-11.0 mmol/L; IFG: FPG 6.1-6.9 mmol/L and 2h-PG <7.8 mmol/L; normal glycaemia: FPG <6.1 mmol/L and 2h-PG <7.8 mmol/L] (9). The posttransplant values in Paper 2 were analyzed in venous whole blood, and classified accordingly using the WHO criteria for whole blood (DM: fasting glucose ≥6.1 mmol/L and/or 2-h glucose ≥10.0 mmol/L; IGT: fasting glucose <6.1 mmol/L and 2-h
glucose 6.7–9.9 mmol/L; IFG: fasting glucose 5.6–6.0 mmol/L and 2-h glucose <6.7 mmol/L; normal glycaemia: fasting glucose <5.6 mmol/L and 2-h glucose <6.7 mmol/L) (97). In Paper 1, the 2003 ADA criteria were used [IFG (FPG 5.6-6.9 mmol/L and 2h-PG <7.8 mmol/L); normal glycaemia (FPG <5.6 mmol/L and 2h-PG <7.8 mmol/L); IGT and DM as in WHO criteria for plasma samples] (98). In the 2010 ADA classification, these criteria have been extended to also include the possibility to diagnose DM by A1C≥6.5% in patients with normal red cell turnover (12). Although the latter is often not the case in patients with ESRD, the 2010 ADA criteria were briefly explored in the present summary of Paper 1 (not in the accepted article). Patients having DM, IGT or IFG were defined as having hyperglycaemia.

7.4 Other investigational procedures

7.4.1. Paper 1

In addition to the pretransplant OGTT, other data were extracted from the referral and work-up forms: age, gender, ethnicity, body-mass-index, renal diagnosis, prednisolone use, dialysis mode and duration, haemoglobin, albumin, total cholesterol, A1C, FPG and 2h-PG.

7.4.2. Paper 2

In addition to the pre- and posttransplant OGTT, several correlates of posttransplant renal function were assessed. Measurements were performed at the 10-week follow-up at our centre, and included a measurement of GFR (\(^{51}\)Cr-EDTA methods), haemoglobin (cyanomethemoglobin methods), phosphate (ammonium molybdate methods), intact PTH (chemiluminescence immunoassays), ionised calcium (potentiometric methods), urea (urease-glutamate dehydrogenase methods) and creatinine (creatinate methods).

7.4.3. Paper 3

On the morning of day 2, fasting blood samples were drawn for baseline prednisolone and routine laboratory tests. Oral prednisolone was then given according to each subject’s regular regimen. Prednisolone was measured at 0, 0.5, 1, 1.5, 2, 4, 6, 8, and 12 hours. At each time point, the total prednisolone concentration was analyzed with a specific radioimmunoassay, in which serum was incubated with anti-prednisolone antibodies and [\(^{3}\)H]-labelled prednisolone.
Bound and unbound prednisolone were separated in a charcoal suspension, followed by a measurement of radioactivity in the supernatant. The protein binding of prednisolone was studied by equilibrium dialysis, in which serum was dialyzed against a \(^{[3]}\text{H}\)-prednisolone containing buffer. The fraction of protein-bound prednisolone at equilibrium was calculated as \((\text{serum dpm} - \text{buffer dpm})/\text{serum dpm}\) (dpm=disintegrations per minute of \(^{[3]}\text{H}\)).

7.5. Statistical analysis

7.5.1. General considerations

7.5.1.1. Missing data

All clinical research is potentially subject to information bias due to missing data. Participants may refuse to perform a particular test, and investigators may omit a test, intentionally or not, or forget to record a test result. The lack of data usually follows patterns, although these are often unapparent. Measurements of glucose, for instance, may be more often missing in young compared to older subjects. This can be due to a perception that such measurements are less important among young patients, since they have a lower prevalence of DM. This creates a situation where the missing glucose data are systematically different (in this case lower) than the observed data, leading to potential bias. Failure to recognize the mechanisms leading to missing data can have a large impact on the internal validity of the results in a study.

Until recently, the presence of missing data has been difficult to handle statistically. All statistical models require complete data sets, and subjects with incomplete data have commonly been excluded from the analysis (complete case analysis). Unfortunately, this approach is only satisfactory when there is no underlying pattern explaining the lack of data (i.e. data are missing completely at random, MCAR) (99;100). This is rarely the case, however, and complete case analysis is normally inadvisable unless the proportion of patients having missing data is below 5-10% (99;101). When missing data are not MCAR, they can follow one of two patterns, namely missing at random (MAR) or missing not at random (MNAR) (99-101). The presence of a MAR pattern indicates that, although the missing data may be systematically different from the observed data, this difference can be explained by other variables in the data set. For instance, if missing glucose values are lower than the observed glucose values, this may be related to the age of patients. Young patients may be less likely to have glucose measured, and simultaneously happen to have lower glucose...
levels. In this example, systematic differences for missing glucose can be explained by age, and MAR is present given that both glucose and age are recorded variables in the data set. The MNAR pattern differs from the MAR pattern in that the systematic differences can only be explained by factors that are not recorded in the data set. For instance, an MNAR pattern would exist if only young patients have missing data, and we have not recorded the age of the patients. MAR and MNAR patterns can not be distinguished mathematically, and the presence of a MAR pattern can only be assumed, not proved. However, the likelihood that a MAR pattern is present, i.e. that the observed variables explain why data are missing, increases with the number of recorded variables in a data set (100;102).

7.5.1.2. Multiple imputation

When a MAR pattern can be assumed present, and the proportion of subjects having missing data is not too high (>60% has been suggested), data can be analyzed using multiple imputation (99;100;102;103). This is a statistical procedure designed to minimize the bias introduced by missing data, and has become an integral part of most statistical computer programs. Imputation refers to the replacement of missing data with statistically probable values. This replacement can prevent investigators from having to exclude subjects with incomplete data, and thereby enable the statistical power to be maintained as compared to complete case analysis. The values used to replace missing data are generated using regression models taking all other variables in the data set into consideration. For instance, if age, body mass index (BMI) and blood pressure, but not glucose, is known for a patient, the glucose can be estimated based on a combination of the patient’s age, BMI and blood pressure, as well as the age, BMI, blood pressure and glucose values of all other participants. However, since the estimated values are only based on probabilities, some of the estimated values may be far from the “true” unobserved value, and may therefore introduce additional bias. For this reason, it is preferable to do multiple imputations, rather than a single one. By doing multiple imputations, the generated values are allowed to differ from one imputation to the next, reflecting the inherent uncertainty with which missing data are replaced. In multiple imputation, one therefore ends up having a number of different copies of the original data set, in which missing data have been replaced by different, and yet statistically probable values from one imputed data set to the next. To capture the uncertainty involved when replacing missing values, it is often recommended that ≥10 imputed data sets are created (99;102;103). Statistical analyses can then be performed separately on each imputed data set, upon which
the separate results from each data set are pooled to yield the final result of multiple imputation. The strength and final aim of multiple imputation is therefore not to replace missing values per se, but rather to create a more valid overall impression of the associations between variables than has been possible with traditional methods for handling missing data.

7.5.2. Final analyses

In papers 1 and 2, 45% and 28% of patients had \( \geq 1 \) missing data entry, respectively. In both papers, the presence of a MAR pattern for missing data was found to be probable. In Paper 3, the prevalence of missing data was low (<10%). Multiple imputation was therefore applied prior to statistical analysis in Papers 1 and 2 as detailed in each respective paper.

Data are presented as mean (standard deviation), median (interquartile range) or frequencies (%). Groups were compared using parametric (paired or unpaired t-test; ANOVA), rank-based (Mann-Whitney; Wilcoxon signed rank; Jonckheere-Terpstra) or \( \chi^2 \) methods, with Fisher’s exact methods as appropriate (2x2 tables with expected cell frequencies <5). Bonferroni corrections were used for multiple comparisons. Continuous variables were compared using parametric (Pearson's r) or non-parametric correlations (Spearman's Rho). For all analyses in the present work, two-tailed p values <0.05 were considered statistically significant. Analyses were implemented in SPSS versions 13 and 17.

7.5.2.1. Paper 1

Patients were classified as having undiagnosed DM, IGT, IFG or normal glycaemia. The prevalence of undiagnosed hyperglycaemia or DM was compared to the general population by indirect standardization methods, applying the DECODE prevalence rates for each gender and 10-year age interval (104). The standardized morbidity ratio (SMR) is reported, and a value >1 indicates a greater burden of disease. Fasting and postprandial classifications were cross-tabulated to visualize the prevalence of hyperglycaemia by each modality. Receiver operating characteristic (ROC) analysis was applied among patients having a non-diabetic FPG in order to assess the accuracy of FPG or A1C for predicting a diabetic 2h-PG. To assess the impact of the 2010 ADA classification for DM, ROC analyses were subsequently repeated among patients having non-diabetic values for both FPG and A1C (<6.5%). Specific methods relating to the ROC analyses are detailed in the Paper 1 article.
7.5.2.2. Paper 2

Multiple logistic regression was used to study risk factors for PHYG. Explanatory variables were selected a priori, and included the pretransplant OGTT result (FPG or 2h-PG), metabolic correlates of posttransplant renal function (urea, phosphate, ionized calcium, PTH, GFR), as well as confounders and known risk factors. The contribution of pretransplant glycemia to the occurrence of PHYG was assessed using the log likelihood method for comparing models including or excluding pretransplant glucose data.

7.5.2.3. Paper 3

Multiple linear regression was used to study risk factors for reduced glucose tolerance. Explanatory variables were selected a priori and included the AUC of unbound prednisolone, alternatively the daily prednisolone dose, and other established risk factors.
8. RESULTS

8.1. Paper 1

Undiagnosed hyperglycaemia before RTx was observed in 326 (37%) patients [WHO criteria; 72 DM (8%), 230 IGT (26%), 24 IFG (3%)]. A minority of the patients were receiving dialysis treatment prior to the referral (n=349, 39%). These patients had lower FPG and A1C levels as compared to predialytic patients [5.1 vs. 5.2 mmol/L and 5.4 vs. 5.6 %, respectively).

The diagnosis of DM required an OGTT in 56 of 72 cases (78%), as only 16 of 72 patients (22%) with diabetic glycaemia were diabetic by FPG criteria alone. The majority (78%) of the diabetic patients would therefore be classified as non-diabetic if the OGTT were to be omitted. Some 50% of the diabetic patients would also have been classified as non-diabetic if the OGTT were to be restricted to patients having IFG.

In the ROC analysis, FPG was an accurate predictor of a diabetic 2h-PG (AUC 0.734, 95% CI 0.674-0.795). When restricted to patients having FPG 5.1-6.9 mmol/L, the OGTT identified ≥80% of patients with a diabetic 2h-PG alone (49 of 56 patients; 88%). Overall, this stepwise procedure (FPG followed by OGTT in selected patients) identified 65 of 72 patients with undiagnosed DM (90%; 16 by FPG, 49 by 2h-PG), and required an OGTT in 463 of 873 patients (53%) with a non-diabetic FPG. These results were consistent among the predialytic patients as well as in patients on dialysis, and were essentially the same when ROC analyses were restricted to patients having both FPG<7.0 mmol/L and A1C<6.5% (ADA 2010 criteria).

In contrast to FPG, ROC results indicated that A1C could not be used as a general tool to identify patients who should proceed to a diagnostic OGTT (AUC 0.578, 95% CI 0.482-0.673). The accuracy improved when studied in predialytic patients alone, but did not surpass that of FPG even here [AUC 0.689 (95% CI 0.586-0.791) vs. 0.710 (95% CI 0.624-0.795); A1C vs. FPG]. The gain from using A1C alone or combined with FPG was negligible whether patients had or had not been started on dialysis. The ROC results for A1C were consistent when restricted to patients having both FPG<7.0 mmol/L and A1C<6.5%.
8.2. Paper 2

Ninety-three patients had PHYG (31%; 2 IFG, 52 IGT, 39 DM), most of whom were diagnosed by 2h-PG at follow-up (67%; 52 IGT, 10 DM). The same proportion of patients was found to have hyperglycaemia at the time of pretransplant referral (n=93; 5 IFG, 65 IGT, 23 provisional DM; unpublished data).

With each increment in pretransplant FPG or 2h-PG, the risk for PHYG increased in a continuous manner (p<0.001). Accordingly, pretransplant FPG and 2h-PG were higher among patients later diagnosed with PHYG than in patients having normal glycaemia after RTx. In PHYG patients, glucose handling deteriorated posttransplant (FPG 5.2 vs. 5.8 mmol/L; 2h-PG 7.4 vs. 8.8 mmol/L; pretransplant vs. posttransplant, respectively; all p<0.001). The opposite was observed in patients having normal posttransplant glycaemia, where glucose handling improved rather than deteriorated (n=208; FPG 4.9 vs. 4.8 mmol/L; 2h-PG 6.2 vs. 5.2 mmol/L; all p<0.001). A similar result was seen for 2h-PG in the population as a whole.

Pretransplant levels of FPG or 2h-PG were further predictive of posttransplant urea levels. Patients with PHYG had higher levels of urea, but similar levels of all other parameters related to renal function as compared to normoglycaemic patients. Increments in urea were associated with a continuous increase in the risk for hyperglycaemia (p<0.001), and this was consistent at low, medium as well as high levels of GFR. Urea levels were related to some correlates of hyperglycaemia (old age, prednisolone dose and acute rejections), but also to male gender and correlates of renal function (phosphate, haemoglobin and GFR; all p<0.05).

Increments in pretransplant glycaemia and posttransplant urea levels remained associated with PHYG after adjustment for important confounders and risk factors. Omission of pretransplant glycaemia (FPG or 2h-PG) from the statistical models did not affect the role of urea, but significantly reduced the models’ ability to discriminate between posttransplant hyper- and normoglycaemia. Comparison of models containing either FPG, 2h-PG or both provided a statistical indication that PHYG was more effectively predicted by pretransplant values of 2h-PG as compared to FPG.
8.3. Paper 3

The median daily prednisolone dose was 10 mg (0.15 mg/kg). Due to gender differences in body weight, the weight adjusted daily prednisolone dose was higher among females as compared to males (0.17 vs. 0.14 mg/kg). Gender differences were also observed regarding the pharmacokinetics of prednisolone. Similar to the weight adjusted prednisolone dose, the $C_{\text{max}}$ and AUC of total prednisolone were higher in females as compared to males. Despite the higher weight adjusted prednisolone dose, however, total prednisolone clearances were lower among female participants. This coincided with a poorer renal function as compared to male subjects. Females had a lower 2h-PG as compared to males, although most results were well within the normal range for both genders. Accordingly, hyperglycaemia was a relatively rare event in this study, and was only present in 15 patients (14%; 3 DM, 11 IGT, 1 IFG).

Reductions in glucose tolerance (increased 2h-PG or glucose AUC) correlated with an increasing AUC of unbound prednisolone, but not with the dose (mg/d or mg/kg/d) or any of the remaining pharmacokinetic parameters of prednisolone. These results were present overall and among males, but not in females, where glucose tolerance seemed entirely unrelated to prednisolone. Based on this observation, an interaction term (female*AUC prednisolone) was studied to explore whether prednisolone could be less diabetogenic in females as compared to males. In crude analysis, there was some indication that this could be the case (p=0.035).

The crude associations between glucose intolerance and prednisolone were verified in multiple regression analyses adjusted for several known confounders. The AUC of unbound prednisolone, but not the prednisolone dose, was associated with glucose intolerance. Once again, this result could not be observed among females. The interaction term for gender specific diabetogenic effects was insignificant in multiple regression analysis and therefore discarded. In addition to the AUC of unbound prednisolone, glucose intolerance was independently associated with old age, elevated triglyceride levels and the use of furosemide.
9. DISCUSSION

9.1. Limitations

9.1.1. Definition of hyperglycaemia

Although the OGTT has been emphasized for defining hyperglycaemia in the present work, there is controversy as to whether FPG, 2h-PG or A1C should be used for this purpose. Fasting and postprandial hyperglycaemia seemingly have different pathogeneses (20) and prognoses (105). On one hand, the 2h-PG appears to have superior accuracy for predicting macrovascular endpoints (106). The OGTT could thus have particular relevance in the RTx setting, where macrovascular disease takes a high toll on patient survival. This supported the use of the OGTT in the present work. On the other hand, FPG and A1C are not inferior to 2h-PG for delineating the risk of microvascular complications (107). To the contrary, 2h-PG may be less convenient and repeatable as compared to A1C and FPG, and tends to overestimate glycaemia in single measurements (108). There is also a possibility that abnormalities in gastric motility contributed to this end in the present work. Delays in gastric emptying predispose to increments in 2h-PG (109), and are common in patients with CKD (110). The use of single OGTT data may thus have created flawed estimates in our studies.

The discrete levels of glucose that should define abnormality are also debatable. The risk of diabetic complications begins to rise at glucose levels below those currently defined as diabetic, and appears to increase continuously with increasing glucose levels. The incidence of retinopathy begins to increase around FPG 6.1 mmol/L, A1C around 6.5%, or 2h-PG in the range between 8 and 10 mmol/L (107). For macrovascular outcomes, the thresholds indicating an increased risk have been less consistent, although it seems that current definitions of intermediate hyperglycaemia predict adverse outcomes in both the general (106) and RTx populations (4;66). As the body of evidence expands, current diagnostic thresholds will presumably be subject to renewed scrutiny in the future. Categories of glycaemia should thus be interpreted as a continuum of glycaemic excursions rather than discrete entities.

9.1.2. Epidemiological methods

The present work is primarily based on cross-sectional data. Such data contain no information on how outcomes are generated over time, which is important for studying whether causality
exists between two associated variables (111). Consequently, cross-sectional data can not be used for studying the causal mechanisms of disease, and the present work should be interpreted accordingly. However, cross-sectional data are useful for other purposes. They are effective for studying the prevalence of disease (Paper 1), and also allow hypothetical associations to be explored with a relatively small demand on resources (Paper 2 and 3). Such associations may then be studied longitudinally to assess whether an observed association could be causal in nature. This was only partly possible in the present work (Paper 2).

Cross-sectional data are observational. Observational studies typically provide poorer information than experimental studies, since in observational studies, it is often impossible to control for all factors that affect the outcome. This can result in bias (systematic error). Bias can lead researchers to reject hypotheses that are true (type I error), or maintain hypotheses that are false (type II error). A bias that shifts the estimate towards the estimate stated in the null hypothesis (i.e. the ‘null’) is traditionally more conservative than a bias that shifts the estimate away from the null. Selection bias arises if the associations between variables are different in patients included in a study as compared to the target population. Information bias arises if the available information differs in quality between patients with vs. without a certain characteristic. This includes misclassifications, which can be differential or non-differential. A misclassification is differential if the probability of being misclassified follows a pattern. This is often the case for missing data, which fall into this category. If there is no pattern, the misclassification is non-differential. Finally, confounder bias results from a failure to consider important variables (confounders) that are known to affect each of two associated variables.

In addition to bias, results can be affected by random errors. Random errors can be reduced using appropriate statistical methods and adequately sized populations. Such strategies are, with a few exceptions (e.g. multiple imputation), not helpful for reducing bias. To reduce bias, careful study designs are considerably more important. In the present work, random errors could be handled statistically, and probably had minor influence on results. Nonetheless, some degree of bias is likely to have been present.

9.1.2.1. Paper 1

The potential for selection bias was relatively small in this study. All referrals for RTx in Norway are evaluated at our centre, and each referral received within the study period was considered specifically. Results are likely to be representative of non-diabetic RTx candidates in Norway, but they should not be inferred to non-Caucasian populations. It is otherwise
possible that referring nephrologists may have failed to inform of a prior diagnosis of DM in some patients, and that the prevalence of undiagnosed hyperglycaemia was overestimated. On the other hand, a normal OGTT at referral may reduce the reporting of previous diabetic FPG results. This could in turn lead to the inclusion of presumed normoglycaemic patients, who in reality should have been excluded due to prior DM. This could falsely reduce the diagnosed prevalence of hyperglycaemia. The overall direction of selection bias was therefore unclear.

Information bias was suggested by missing data and the imprecise reporting of the glucose test materials. Missing data were examined by multiple imputation and did not seem to influence results. Only a small number of OGTTs (n=81) were reported measured in whole blood. If the true number were higher, the prevalence of undiagnosed hyperglycaemia may be underestimated as presently reported (since whole blood results should be multiplied by a factor of 1.11) (96). In spite of this, the observed prevalence was high.

9.1.2.2. Paper 2

This study included some patients in whom undiagnosed DM was detected at referral. Although none of these patients were commenced on glucose lowering drugs before RTx, some of them may have been subjected to non-pharmacological intervention while awaiting RTx. This may have altered glucose levels, and thereby biased the role of pretransplant glycaemia. However, such interventions would tend to lower glucose levels, and move bias towards, rather than away from the null. Due to the small number of non-Caucasian subjects in our study, inference to non-Caucasian populations should be done with caution.

A number of patients were lost to follow-up (withdrawal bias). These patients may have been sicker and had different levels of glucose, urea and other parameters compared to the included patients. Results may thus have been different in these patients. Other types of information bias were probably also present. Specifically, the reasons why pretransplant glucose data were missing in a number of patients was not entirely clear. It seems likely, however, that multiple imputation reduced the information bias associated with missing observations. Efforts were made to handle confounding by multivariable regression analysis.

9.1.2.3. Paper 3

In this study, various mechanisms may have lead to selection bias. Our findings are representative of patients selected for not having DM, and could have differed if diabetic
glucose levels were more prevalent. Results may also have been different among the 56 patients who were excluded for not having consented to the OGTT. Most patients were studied a long time after RTx, and results could have been different at a shorter time posttransplant. The sum of selection biases may either have over- or underestimated our findings. Other types of bias (information, confounding) probably played a lesser role. The study was consistently planned and executed, and confounding was sought addressed using multivariable analysis.

9.2. Interpretation of results

The last few decades have seen an increased scientific and clinical interest in the development of glucose abnormalities after RTx. These abnormalities, and NODAT in particular, have serious consequences affecting both patient and graft survival (57;60-62). Many studies have been performed to examine the incidence of NODAT, and to identify the risk factors that lead to the development of this complication.

The reported incidence of NODAT has varied widely between studies (55). While this partly reflects differences in the distribution of risk factors between populations, the variability is also attributable to differences in criteria used to define diabetes. It is encouraging that standard criteria for diagnosing NODAT have become established in recent years (70). Unfortunately, however, the same precision is rarely achieved when patients are classified and included in studies according to pretransplant status. Only few studies on NODAT have formally assessed pretransplant glucose tolerance (79-82). Instead, patients with pre-existing DM are typically excluded based on imprecise dichotomous data from charts or registries (6;57). This can alter the prevalence estimates for pre-existing DM and also the incidence of NODAT. The natural history of glucose tolerance in relation to RTx is most reliably studied using a formal assessment of glycaemia both pre- and posttransplant. In the present work, such measurements were available before as well as after RTx.

9.2.1. Glycaemia before renal transplantation

Undiagnosed hyperglycaemia and DM were prevalent in Norwegian kidney transplant candidates (Paper 1). Among all apparently non-diabetic patients considered eligible for RTx during the last 7 years, undiagnosed hyperglycaemia and DM were present in 37% and 8% of the patients, respectively, at the time of referral. The prevalence of hyperglycaemia would
have been even higher if the 2003 [n=402 (45%); 72 DM (8%), 230 IGT (26%), 100 IFG (11%)] alternatively 2010 ADA criteria were to be uniformly applied [n=422 (47%); 112 DM (13%), 214 IGT (24%), 96 IFG (11%)]. Forty-nine (6%) patients had an A1C equivalent to DM by the new ADA criteria (≥6.5%), of whom nine (18%) also met the standard DM criteria for glucose measurements. Consequently, the sensitivity of the A1C criteria for detecting DM by the glucose criteria was 13% (9 of 72 DM detected; unpublished data), indicating that A1C and plasma glucose criteria would identify very different subsets of patients before RTx.

The observed prevalence of both undiagnosed hyperglycaemia and DM in the pretransplant population is higher than that of an age and gender standardized general European population from the DECODE study [SMR 1.60 (95% CI 1.43-1.79) and 1.47 (95% CI 1.16-1.85); undiagnosed hyperglycaemia and DM, respectively] (104). This result has not previously been reported in a large pretransplant population. It is unclear to which extent our findings could be explained by uraemic IR or other factors (e.g. differences in ethnicity, body composition or cardiovascular comorbidity). With one exception (haemoglobin), we had no data on the metabolic factors proposed to influence insulin sensitivity in uraemia, such as calcium, phosphate, PTH, urea, protein turnover or metabolic acidosis. Nonetheless, the general result in our study is in agreement with the previous observation that the incidence of DM is higher among ESRD patients as compared to the general population (6).

The OGTT seemed to be of particular importance for identifying undiagnosed hyperglycaemia before RTx. Using WHO criteria, some 75% and 80% of all patients having undiagnosed hyperglycaemia and DM, respectively, would have been missed if the OGTT had not been performed (Paper 1). These figures are higher than those found in the general population, where only 40% and 30% of patients would have been missed, respectively (105). In morbidly obese individuals, FPG alone identified 80% of patients with undiagnosed DM (112). This could suggest that in uraemic patients, to a larger extent than in many other populations, hyperglycaemia is primarily a postprandial phenomenon. A similar observation has been made by others (24;113;114), but until the present work, the predisposition for postprandial hyperglycaemia has not been demonstrated in a large population of ESRD patients. The explanation for this predisposition could involve a variety of ESRD related factors. First, elevations in FPG may be concealed by the presence of fasting hyperinsulinaemia in ESRD (32). Mild cases of hyperglycaemia may therefore remain undetected unless formally tested using an OGTT. Second, uraemic subjects are characterized by peripheral IR (5), and have a reduced uptake combined with increased production of glucose by the liver after an oral (114) or intravenous glucose challenge (115). In subjects
without renal disease, peripheral IR predisposes to postprandial hyperglycaemia (20), which in turn may reveal or promote β cell dysfunction. This mechanism could be particularly pronounced in uraemic subjects, where peripheral IR is near universal.

It is current practice in Norway to perform a pretransplant OGTT in all kidney transplant candidates without a prior diagnosis of DM. International guidelines propose a similar strategy (116). These approaches ensure a high diagnostic accuracy for pretransplant DM, but are also time consuming and potentially costly to incorporate into clinical practice. We therefore explored the accuracy of FPG as a tool to identify subjects likely to have a diabetic OGTT before RTx. With a minimal (10%) loss in the DM detection rate, we observed that restricting the OGTT to subjects with FPG 5.1-6.9 mmol/L could reduce the number of patients requiring an OGTT by some 50% (Paper 1). This could significantly reduce the time and resources required to perform the OGTT. Admittedly, however, the clinical significance of these findings remains unclear. First, it may not be sensible to identify DM pretransplant if the excess risk for PHYG is also high among patients with pretransplant IGT or even lower glucose levels. We (Paper 2) and others (79-82) found that the risk of PHYG begins to rise at non-diabetic levels of pretransplant glycaemia. Second, it is unknown at which thresholds and to what extent pretransplant glycaemia predicts the primary outcome of interest, namely the risk of posttransplant complications such as impaired patient and graft survival. There is also a need to clarify whether patients with vs. without pretransplant hyperglycaemia draw different benefits from receiving a calcineurin inhibitor with a lower risk of PHYG (75).

Based on the increasing focus on the use of A1C to diagnose DM (12), we explored the role of A1C before RTx. A1C seemed to be of limited advantage both for diagnosis and case finding of DM in RTx candidates. First, the overlap was poor between A1C and standard glucose based criteria, as the majority (82%) of patients with DM by A1C had non-diabetic levels of both FPG and 2h-PG. Second, A1C seemed inappropriate for predicting a diabetic OGTT. This result is in contrast to those from the general population and early posttransplant period, where the ability of A1C to predict a diabetic 2h-PG seemed comparable to that of FPG (117;118). It is likely that several mechanisms contributed to these discrepancies. First, erythropoietin use, iron supplements, and blood loss during hemodialysis may have increased the turnover of red blood cells, and thereby falsely reduced A1C levels (119-121). In agreement with these reports, dialyzed patients in our study population were found to have lower levels of A1C. Second, the association between A1C and glycaemia is altered in uraemic patients due to the increased formation of carbamylated haemoglobin (cHb).
Carbamylation and glycation are competing processes occurring at similar binding sites on the haemoglobin molecule (122). Third, A1C and cHb have similar isoelectric points, resulting in a tendency of certain A1C assays, charge-based ones in particular, to report a result that in fact is a combined concentration of A1C and cHb (123;124). This suggests that A1C may be less effective as a marker of glycaemia in patients with ESRD, particularly in patients on dialysis, as compared to other populations. Our findings therefore align with the general reservations made by the ADA that A1C should not be used to diagnose DM in patients with abnormal red cell turnover (12).

9.2.2. Glycaemia short term after renal transplantation

The prevalence of PHYG and posttransplant DM at 10 weeks posttransplant were 31% and 13%, respectively. Similar to the pretransplant setting, roughly 70% of the patients with WHO defined hyperglycaemia required an OGTT to be identified. This is in general agreement with other studies reporting a higher diagnostic yield for PHYG using the OGTT as compared to FPG alone after RTx (56;58;59;125). Three of these studies included an OGTT performed within a similar time frame as compared to our work (2-3 months posttransplant), and reported the incidence of NODAT to vary between 12% and 31% (56;58;125). None of the studies performed a formal pretransplant assessment of glucose tolerance, however, making it unclear to which extent the observed cases of NODAT were truly incident. In our experience, exclusion of the patients with provisional DM before RTx (n=23) reduced the apparent incidence of DM from 13% to roughly 10% (i.e. a 30% overestimation of the incidence). This suggests that the pretransplant OGTT is important to evaluate the true incidence of NODAT.

The presence of PHYG was highly reflective of pretransplant glycaemia, especially when the latter was assessed using postprandial glucose. To our knowledge, this has not been observed previously. Our results may be interpreted in the light of two reports indicating that in non-diabetic RTx candidates, the development of PHYG is predicted by pretransplant β cell dysfunction rather than IR (80;82). It is thus conceivable that pretransplant 2h-PG, to a larger extent than FPG, was able to identify subjects with impaired pretransplant β cell function in our study. This possibility is supported by a recent report, where postprandial hyperglycaemia tended to reflect insulin hyposecretion rather than resistance in RTx candidates (113).

An ability of 2h-PG to disclose β cell dysfunction could have clinical implications. First, by exposing the presence of β cell impairment, an elevated 2h-PG may identify subjects in whom the progression to overt DM is relatively imminent even before RTx. In the general
population, the combined presence of IR and insulin hyposecretion signifies a high short term risk of developing overt type 2 DM (126). For some patients in our study, PHYG may thus have resulted from a process that was already far advanced before RTx. Second, and possibly more important, an elevated pretransplant 2h-PG may have indicated a susceptibility to the diabetogenic effects of immunosuppressive drugs. Calcineurin inhibitors seem to impair β cell function (76;127), and could therefore be particularly unfavourable in patients who are already on the brink of β cell failure prior to reaching RTx (77).

In patients who did not develop PHYG, both fasting and postprandial glucose improved after RTx. This may seem difficult to reconcile with the fact that all patients had been exposed to a significant surgical trauma and diabetogenic immunosuppressive drugs. It is nonetheless conceivable that the improvement in renal function after RTx in these patients may have caused an amelioration of the IR associated with CKD. However, IR has been found to diminish (80), increase (69) or remain unchanged after RTx (82). This illustrates that glycaemia after RTx is influenced by many other factors than renal function, such as immunosuppressive drugs. We did not measure insulin, and can only speculate on the factors that may have lead to an improvement in glycaemia in our normoglycaemic patients. Diminished IR, a superior β cell function, or a combination of both may have been operative.

The levels of urea and glucose were strongly associated after RTx. In accordance, patients with PHYG had significantly higher levels of urea as compared to normoglycaemic patients. This finding has not been described before, and can have different interpretations. Urea may have reflected renal impairment in patients with PHYG, since renal failure reduces the elimination of urea and is also associated with IR (30). We believe, however, that this was of little importance in our study. Urea and glucose were related at all levels of GFR, and GFR was similar whether patients were hyper- or normoglycaemic. This is in agreement with the recent observation that, in comparison to the pretransplant setting (30), GFR and IR correlate poorly after RTx (128). Alternatively, urea could represent an association between protein catabolism and glycaemia. As illustrated in Figure 2, several mechanisms could be operative.

The glycaemic impact of protein catabolism may be extended to involve urea directly (Figure 2). An increasing body of evidence from human and animal studies lends credibility to the hypothesis that urea may in itself be diabetogenic. Glucose tolerance is temporarily normalized by dialysis in patients with ESRD, suggesting that uraemic IR involves a dialyzable solute such as urea (34;45;129). Urea levels correlate with IR in patients with CKD (130) and are lowered by low-protein diets, which have insulin-sensitizing effects in these patients (131). Administration of urea has been found to reduce glucose disposal in healthy
males (132), in stable CKD patients (36) as well as in mice (133). In the latter study, urea alone caused a similar degree of IR in healthy mice as that seen in nephrectomized controls.

**Figure 2.** Associations linking protein and glucose metabolism

This diabetogenic effect was mediated by a urea-induced generation of reactive oxygen species, which resulted in impaired signalling downstream of the insulin receptor, and subsequently lead to IR (133). The proximal mechanism of urea-induced IR is likely to involve carbamylation, which describes the modification by urea-derived cyanate of protein or amino acid structure and function. Carbamylated amino acids reduce insulin sensitivity in vitro (37), and were proposed to have mediated the effect of urea on the generation of oxidative stress (133). These observations could provide an explanation for the association between urea and PHYG in our study, and also suggest that protein catabolism is an important component in the pathogenesis of not only pre-, but also posttransplant hyperglycaemia.

With the possible exception of urea, PHYG was poorly correlated with parameters reflective of renal function. The GFR was similar whether patients did or did not have PHYG. Nonetheless, patients with PHYG appeared to have a slightly higher GFR when adjusting for age. This difference could represent a degree of hyperfiltration in our PHYG patients, which has been proposed to indicate early graft injury resulting from hyperinsulinaemia (139).
of calcium, phosphate, PTH or haemoglobin displayed an association with glycaemia, despite a number of reports suggesting that such associations might exist. Elevations in calcium have been associated with IR among non-diabetic elderly males (50). Low phosphate levels are common after RTx, and have been associated with IR in subjects without renal disease (43;140;141). Hyperparathyroidism has been found to impair β cell function in animals (49), while in humans, parathyroidectomy has been followed by increased (46;48), unchanged (42) or even reduced (52) insulin secretion. Correction of anaemia by erythropoietin has been found to ameliorate IR in hemodialysis patients (41). A number of factors could not be accounted for in our study, however, making it difficult to conclude that no associations were present. We did not measure IR, and had no data on vitamin D or FGF-23, both of which are confounders for the calcium-phosphate-PTH axis after RTx (142;143).

9.2.3. Glycaemia long term after renal transplantation

PHYG is typically manifested early after RTx, but often tends to regress with tapering of immunosuppression. In spite of this, the prevalence of PHYG seems high even long term after RTx. The OGTT-determined prevalence of PHYG in stable RTx recipients without known DM, most of whom were Caucasians, recently amounted to some 30-50% at 2-6 years after RTx (144-146). Similar to these reports, we performed an OGTT in stable RTx recipients without known DM at 5 years after RTx (Paper 3), but observed PHYG in less than 15% of our patients. Since our patients used twice the prednisolone dose that is commonly used today, it is tempting to speculate that CNI therapy, which was not applied in our study, may have contributed significantly to PHYG in the other reports. Early impairments in β cell function are associated with CNI therapy (76;77), and appear to reduce the likelihood that NODAT will be reversible over time (68;69;146). Alternatively, differences in age, BMI, GFR or other factors may have been operative. In any case, the discrepancy was not related to differences in the diagnostic criteria applied, since our findings were the same whether WHO or ADA criteria were used (not shown).

9.2.4. Role of prednisolone in glycaemia after renal transplantation

A large study recently reported that the diabetogenicity of prednisolone depended on the type of concomitant CNI therapy prescribed (147). In this study, the risk of NODAT increased with increasing prednisolone doses, but only in patients using tacrolimus. However, the
DIRECT study reported the very opposite finding, namely that prednisolone was only diabetogenic in patients using CsA (75). It is therefore unclear how and to what extent the interpretation of prednisolone effects might be confounded by CNI therapy (84). Until shown otherwise, CNIs and prednisolone are both considered important risk factors for NODAT (7;55;71). Despite the co-medication with CNI and tapering of steroid doses, prednisolone seems to be important even long term after RTx. This is suggested by most (144;148), although not all (145) reports addressing PHYG in stable RTx patients without known DM.

Contrary to the majority of current studies, we were able to assess the effects of prednisolone with no possibility of confounding due to CNIs. In line with our hypothesis, we observed a linear relationship between the exposure to unbound prednisolone and postprandial glycaemia. Due to the intracellular location of the glucocorticoid receptor (GR), prednisolone effects are thought to be exerted by the unbound drug, and this is likely also the case for steroid-induced hyperglycaemia (78;149;150). In agreement, there was no association between glucose tolerance and the alternative measures of prednisolone exposure. The mechanisms for prednisolone action and relation to hyperglycaemia are illustrated in Figure 3.

Animal studies have indicated that prednisolone may also impair β cell function (78). In humans, short courses of steroid result in an increase in ISec to compensate for the degree of IR. The inability to counteract steroid-induced IR with a sufficient ISec response was the key determinant for whether or not steroid intake resulted in an elevated 2h-PG during an OGGT (151). The contribution of IR and impaired ISec could not be assessed in our study.

We observed a higher total AUC of prednisolone in females as compared to males in our study. This coincided with a higher weight adjusted dose and lower total clearance of prednisolone among females. While the total clearance of prednisolone normally increases with increasing dose (91), we observed that females had a low clearance despite receiving a high dose of prednisolone. This indicates that the difference in clearance was not dose related, but rather reflected other factors, such as GFR, which was lower in females. Aside from this, it is unclear to what extent our results may or may not have reflected gender per se.

Recent observations have nonetheless indicated that the metabolism of glucocorticoids could display gender specific differences. Our finding that prednisolone tended to be more diabetogenic in males than in females may perhaps be taken to illustrate the existence of such differences. The activity and tissue distribution of 11-β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), an enzyme responsible for the local reactivation of cortisol from inactive cortisone (Figure 3), is increasingly considered a key factor in modulating the local availability and metabolic effects of glucocorticoids (152-154). Transgenic mice that
Unbound prednisolone or other glucocorticoids (ligands) bind to the glucocorticoid receptor (GR), which is thereafter phosphorylated to become biologically active. Subsequent effects occur by genomic or non-genomic pathways. In a genomic pathway, the GR-ligand complex enters the nucleus to interact with the DNA strand, leading to activation (transactivation) or repression (transrepression) of gene transcription. It is thought that side effects (e.g. hyperglycaemia) are mainly mediated through transactivation, whereas desirable therapeutic effects primarily occur by transrepression, alternatively by non-genomic pathways, in which the GR-ligand complex modifies inflammatory substances without altering their gene transcription (78;149;150)1).

Transactivation can promote IR by various pathways (78;150)1. In skeletal muscle, proteins transcribed due to prednisolone-induced transactivation can interfere with insulin signalling, leading to IR by a post-receptor defect. Alternatively, the same proteins may induce IR by first altering intra-myocellular protein or lipid metabolism (increased proteolysis and lipolysis). In the liver, prednisolone promotes HGO by inducing IR, by providing substrate for gluconeogenesis (increased proteolysis and lipolysis), or by directly increasing the transcription of rate-limiting gluconeogenic enzymes. Some of these enzymes have GR-ligand sensitive DNA promoter regions, suggesting that steroids have a direct impact on the hepatic production of glucose. In adipose tissue, prednisolone increases lipolysis, mobilizes fat from peripheral to visceral depots, and may alter the expression of adipokines associated with IR. [Schematic figure created by HA Bergrem].
overexpress 11β-HSD1 develop obesity and IR (155). In humans, the activity of 11β-HSD1 is increased in obesity, and inhibition of the enzyme reduces hepatic glucose output in lean type 2 diabetic males (152). Interestingly, the expression of 11β-HSD1 in lean subjects was recently found to be significantly lower in female than in male subcutaneous adipose tissue (153). Since prednisolone is in part converted to its inactive counterpart, prednisone, upon administration (85), and prednisone is a substrate for 11β-HSD1 (85;154), it is tempting to speculate that increased local reactivation of inert prednisone by 11-β-HSD1 in males could be responsible for the observed gender difference in our study.

Prednisolone meets several of the requirements proposed for concentration monitoring strategies (87). In spite of this, therapeutic drug monitoring has not become clinical practice for prednisolone. Due to a short half-life, prednisolone does not reach steady-state using common regimens, and thus requires an AUC measurement. This is time consuming, but may be omitted if a time point can be identified at which the concentration provides a valid estimate for the AUC (limited sampling). A 2-hour post dose concentration was recently found to correlate well with the AUC of total prednisolone in RTx patients (156), but this has to our knowledge not been established for the unbound drug. In addition, the methods required to measure the unbound fraction of prednisolone have not been routinely available. For these reasons, it has been claimed that therapeutic drug monitoring may not be feasible for prednisolone (87). Nonetheless, the monitoring of cyclosporine faced very similar challenges 20 years ago (91). This suggests that the challenges related to prednisolone monitoring are not necessarily overwhelming. As suggested by Steiner (93), the paucity of studies examining the efficacy of concentration guided steroid therapy may partly reflect the relative absence of a commercial counter-advocacy against the withdrawal of steroids. Regardless of the reason for the lack of trials involving steroid monitoring, the variable results of steroid withdrawal studies indicate that our understanding of steroids remains insufficient to determine the best clinical application of steroids after RTx (71). Our findings could therefore strengthen the rationale for measuring prednisolone concentrations in forthcoming studies.
10. CONCLUSIONS

Undiagnosed DM and hyperglycaemia were prevalent in Caucasian RTx candidates without known DM at the time of referral for RTx. The OGTT was paramount to detect pretransplant DM in these patients. Unlike A1C, FPG helped predict the occurrence of a diabetic OGTT. When screening for DM, the pretransplant OGTT may be omitted, with only a small risk of misclassification, if FPG is below 5.1 mmol/L in Caucasian RTx candidates (Paper 1).

The occurrence of PHYG early after RTx was reflective of elevations in glycaemia occurring before RTx, especially when these were evaluated using 2h-PG. PHYG displayed a strong and independent association with posttransplant levels of urea (Paper 2).

The association between prednisolone exposure and glucose tolerance in stable RTx patients was more precisely evaluated using the unbound AUC as compared to the daily dose of prednisolone, at least in males (Paper 3).
11. IMPLICATIONS AND FUTURE PERSPECTIVES

We have proposed a strategy that may well be helpful for identifying undiagnosed DM in Caucasian RTx candidates without prior DM (Paper 1). The clinical importance of this result is nonetheless unclear. It is not known to what extent it is important to detect hyperglycaemia before RTx, let alone whether undiagnosed DM is a more important entity as compared to intermediate hyperglycaemia. We observed that even non-diabetic elevations in pretransplant glucose predicted an increased risk of PHYG (Paper 2). It is therefore possible that even non-diabetic degrees of pretransplant hyperglycaemia can have implications after RTx. Future studies should explore whether or not the risk of adverse posttransplant outcomes begins to rise at a discrete level of pretransplant glucose. These outcomes should include not only PHYG, but also endpoints such as cardiovascular disease and patient and graft survival.

The association between PHYG and urea levels has not been described previously (Paper 2). Although the direction of this relationship is unclear, it could represent a novel link between uraemic and posttransplant hyperglycaemia. Studies should attempt to explore whether pretransplant or serial early posttransplant urea levels are predictive of PHYG. Measurements of protein and insulin metabolism would be of advantage in this context.

We were able to document the proposed association between the exposure to unbound prednisolone and glucose tolerance without CNI interference. Studies should explore whether this relationship is also present during concomitant CNI therapy. An important goal should be to explore the feasibility of a limited sampling strategy for unbound prednisolone. This may subsequently help identify concentration levels associated with improved outcomes.
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