Original Articles

Divided dosing reduces prednisolone-induced hyperglycaemia and glycaemic variability: a randomized trial after kidney transplantation

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ABSTRACT

Background. Prednisolone is a major risk factor for hyperglycaemia and new-onset diabetes after transplantation. Uncontrolled observational data suggest that divided dosing may reduce requirements for hypoglycaemic agents. This study aims to compare the glycaemic effects of divided twice daily (BD) and once daily (QD) prednisolone.

Methods. Twenty-two kidney transplant recipients without diabetes were randomized to BD or QD prednisolone. Three weeks post-transplant, a continuous glucose monitor (iPro2® Medtronic) was applied for 5 days with subjects continuing their initial prednisolone regimen (Days 1–2) before crossover to the alternative regimen. Mean glucose, peak glucose, nadir glucose, exposure to hyperglycaemia (glucose ≥ 7.8 mmol/L) and glycaemic variability were assessed.

Results. The mean ± standard deviation (SD) age of subjects was 50 ± 10 years and 77% were male. Median (interquartile range) daily prednisolone dose was 25 (20, 25) mg. BD prednisolone was associated with decreased mean glucose (mean 7.9 ± 1.7 versus 8.1 ± 2.3 mmol/L, P < 0.001), peak glucose [median 10.4 (9.5, 11.4) versus 11.4 (10.3, 13.4) mmol/L, P < 0.001] and exposure to hyperglycaemia (glucose ≥7.8 mmol/L) and glycaemic variability were assessed.

Conclusions. Split prednisolone dosing reduces glycaemic variability and hyperglycaemia early post-kidney transplant.

Keywords: hyperglycaemia, new-onset diabetes, prednisolone, transplant

INTRODUCTION

Since the advent of solid organ transplantation, glucocorticoids have been a core component of immunosuppressive regimens [1, 2]. The short elimination half-life of prednisolone (3 h) dictates that daily dosing cannot achieve steady-state plasma levels, and is likely to be exerting little or no effect in the second half of the day [3, 4]. An uncontrolled observational study in 16 kidney transplant recipients suggested that divided daily dosing may produce superior immunosuppressive efficacy with less requirement for diabetes medication [5]. Divided daily dosing may also reduce glycaemic variability, a factor linked with increased oxidative stress and β-cell dysfunction [6]. The preference for daily (or even alternate daily) dosing of prednisolone stems largely from a desire to reduce the suppression of the hypothalamic–pituitary–adrenal axis and the development of Cushing’s syndrome [7]. However, even prolonged use of low dose morning prednisolone (5–10 mg) has been associated with inadequate adrenal response to synacthen, indicating that suppression of the hypothalamic–pituitary–adrenal axis should be anticipated in all kidney
transplant patients on maintenance doses of ≥5 mg [8–10]. This prospective randomized crossover study used continuous glucose monitoring (CGM) to compare glycaemic profiles of kidney transplant recipients taking divided twice daily (BD) versus once daily (QD) prednisolone and to determine whether BD reduces hyperglycaemia and glycaemic variability compared with QD dosing.

### Materials and Methods

This study was undertaken in consenting subjects receiving a kidney transplant at the Royal Melbourne Hospital between October 2011 and August 2012. The study was approved by the Melbourne Health Human Research Ethics Committee.

#### Subjects

Subjects were aged between 18 and 75 years old and had a fasting plasma glucose assessment prior to transplantation to exclude those with pre-existing diabetes.

#### Crossover study design

Using sequentially numbered opaque sealed envelopes generated by a third party, subjects were randomized by study investigators to QD (8:00 h) or BD (50% of total daily dose at 8:00 h and 50% at 20:00 h) prednisolone dosing regimens at the time of transplantation. Prednisolone doses were then adjusted by the transplant team until Week 3 when the total time of transplantation. Prednisolone doses were then administered by a third party, subjects were randomized by study investigators to QD (8:00 h) or BD (50% of total daily dose at 8:00 h and removed after 8:00 h on Day 6. Therefore, 2 days of CGM on each dosing regimen were available for comparison (Days 1–2 versus 4–5).

#### Glucose monitoring

The Medtronic iPro™ 2 CGM system was used with Medtronic Enlite™ glucose sensors placed on the abdomen (Medtronic, Northridge, CA, USA). This system records an average interstitial fluid glucose measurement every 5 min for 6 days. The measurements were blinded to patient and at study conclusion these were downloaded using the Medtronic CareLink® iPro™ software. The CGM was calibrated with four fingerstick capillary glucose tests per day (prior to meals and at bedtime) using the Abbott Optium Xceed™ blood glucose monitoring system (Abbott Laboratories, Abbott Park, IL, USA). The subjects documented capillary blood glucose test results were verified using the Optium Xceed™ device memory. Clarke error grid analysis of Enlite sensor glucose versus gold standard plasma glucose indicates that, over a range of 4.5–22.2 mmol/L, >97% of Enlite sensor readings are within the clinically accurate A + B zones [11]. Between 2.2 and 4.4 mmol/L, over 80% of Enlite sensor readings are within the clinically accurate A + B zones. The sensor is robust and accurate over 6 days of continuous use, and mean time lag compared with plasma glucose is 7.94 ± 6.48 min (95% confidence interval 6.38, 9.50 min) [11].

#### Immunosuppression

Subjects received basiliximab induction and an initial dose of 500 mg of methylprednisolone prior to kidney transplantation. Maintenance immunosuppression comprised of tacrolimus, mycophenolate and prednisolone, with recipients of living donor transplants commencing treatment 2–3 days prior to surgery. Prednisolone was maintained at 20–25 mg daily during the first 4 weeks post-transplant. Target tacrolimus trough levels were 8–12 ng/mL for the first 2 weeks and 5–8 ng/mL by 4 weeks post-transplant.

#### Glycaemic end points

The predetermined study outcomes were mean interstitial fluid glucose, the time and magnitude of peak and nadir interstitial fluid glucose, glycaemic variability and exposure to hyperglycaemia. The latter was defined as the area under the concentration–time curve (AUC) where glucose concentration exceeded 7.8 mmol/L, corresponding with the consensus American Diabetes Association and World Health Organization definition for impaired glucose tolerance after transplantation [12]. Glycaemic variability was assessed using standard deviation (SD), mean amplitude of glycaemic excursion (MAGE), continuous overlapping net glycaemic action (CONGA-1 and -2) and the J-index.

#### Sleep disturbance

As a subjective increase in wakefulness is frequently reported by subjects taking glucocorticoids, the validated Athens

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**Figure 1:** Study protocol. Subjects remained on their randomized prednisolone dosing regimen for Days 1 and 2 before crossover on Day 3 to the alternative dosing regimen. CGM was commenced on Day 1 and removed after Day 5. Day 3 was disregarded as a wash-out period.
Insomnia Scale was used to assess sleep disturbance after two nights on each dosing regimen for all subjects [13]. This scale includes five questions in the domains of sleep induction, nocturnal awakenings, final awakening, total sleep duration and overall quality of sleep. Each question has four possible answers scored between 0 and 3, producing a score range between 0 for no insomnia and 15 for severe insomnia.

**Power calculation**

There were no prior trials comparing glycaemia with QD and BD prednisolone on which to base power calculations. We therefore extrapolated from data reported by the UK Prospective Diabetes Study, which indicated that a reduction in HbA1c of 0.9% was associated with a 12% reduction in any diabetes-related end point and a 25% reduction in microvascular complications [14]. The A1c-Derived Average Glucose (ADAG) study confirmed that a linear relationship exists between estimated average daily glucose and HbA1c [15]. Applying the ADAG equation, a difference in HbA1c of 0.9% correlates with that in blood glucose of 1.4 mmol/L. For power calculations in this pilot study, a clinically important difference in mean glucose was therefore considered to be 1.4 mmol/L; and in the absence superior data, this was also considered to represent a clinically important difference in peak glucose. Assuming this and estimating a SD of 2 mmol/L on changing regimens, 22 subjects were needed for this study (based on 90% power and two-sided \( \alpha = 0.05 \)).

**Data analysis**

Descriptive statistics used were percentages for categorical data, mean ± SD for normally distributed continuous data and median (interquartile range, IQR) for non-normally distributed continuous or ordinal data. Paired and unpaired \( t \)-tests were used to detect the differences in glycaemia and glycaemic variability between BD and QD dosing regimens. The Wilcoxon rank test was used to detect differences in ordinal data between dosing regimens. Area under the glucose concentration–time curve was measured using the trapezoidal rule. Results were calculated using GraphPad Prism version 6.0a for Mac OS X, GraphPad Software (La Jolla, CA, USA). P-values of <0.05 were considered statistically significant.

**RESULTS**

Thirty-four subjects were randomized at the time of transplantation to commence BD (\( n = 17 \)) or QD prednisolone (\( n = 17 \)), with 22 (11 in each arm) completing the study (Figure 2). All subjects were Caucasian, 77% were male and median (IQR) age was 49 (46, 57) years (Table 1). The median prednisolone
Discussion

In the early period after kidney transplantation, acute hyperglycaemia may lead to osmotic symptoms, dehydration and acute renal impairment [16–20], while new-onset diabetes has been associated with a 1.8-fold increase in cardiovascular mortality and a 1.5-fold increase in overall mortality [21]. Prednisolone is a major risk factor for hyperglycaemia and new-onset diabetes after transplantation [12]. The pharmacokinetics of prednisolone are notable for a short elimination half-life (3.0 h), suggesting that divided daily dosing may be more appropriate [3]. To explore this premise, patients without diabetes 3–4 weeks post-kidney transplant participated in a crossover study of BD versus QD prednisolone dosing, with assessment of glycaemic profiles obtained by CGM. BD prednisolone dosing was associated with a lower peak and mean interstitial fluid glucose, a lower exposure to hyperglycaemia (AUC ≥7.8 mmol/L or 140 mg/dL) and less glycaemic variability.

Given the short half-life of prednisolone, it is somewhat surprising that divided daily dosing has not been subjected to more clinical trials. An uncontrolled observational study of patients receiving prednisolone for glomerulonephritis (n = 35) or renal transplantation (n = 16) reported equivalent renal outcomes and a lower total daily dose in patients receiving BD dosing compared with a single morning dose (P = 0.008). Additional findings included the use of less concurrent immunosuppression (mycophenolate and/or cyclosporine, P = 0.017) and a reduced requirement for diabetes medication (P = 0.032) [5]. Although the mean glucose level in our study was only 0.2 mmol/L (3 mg/dL) lower with BD dosing, the glycaemic profiles differed significantly between regimens (Figures 3 and 4). Peak interstitial fluid glucose (median) was 1 mmol/L (18 mg/dL) greater with daily prednisolone administration and the majority of subjects (82%) developed diabetic range results (≥11.1 mmol/L or 200 mg/dL) with this regimen, compared with 50% with BD. Following daily administration at 8:00 h, peak interstitial fluid glucose (median) was detected ~7.5 h later. This is consistent with a study of 40 subjects without diabetes receiving 30 mg prednisolone daily at 8:00 h for an exacerbation of chronic obstructive airways disease, where peak glucose of 11.3 mmol/L (204 mg/dL) occurred at 15:30 h [22]. In contrast, BD prednisolone dosing in the current study (8:00 and 20:00 h) resulted in a peak interstitial fluid glucose (median) at ~15:00 h. Although nadir interstitial fluid glucose (median) was 0.7 mmol/L lower with QD compared with BD prednisolone, overall exposure to glucose levels within an abnormal range (AUC of ≥7.8 mmol/L or 140 mg/dL) was 1.6-fold higher. Following 8:00 h prednisolone administration, median glucose levels were ≥7.8 mmol/L between 10:15 and 23:00 h.

Glycaemic variability has been associated with adverse outcomes and was significantly lower with BD compared with daily prednisolone administration using all indices—SD, MAGE, CONGA-1, CONGA-2 and J-index [23–25]. This may

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**Table 1. Characteristics of kidney transplant recipients studied (N = 22)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49 (46, 57)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>17 (77)</td>
</tr>
<tr>
<td>Prednisolone dose (mg/day)</td>
<td>25 (20, 25)</td>
</tr>
<tr>
<td>Body weight at transplantation (kg)</td>
<td>75 (64, 86)</td>
</tr>
<tr>
<td>Body weight at study commencement (kg)</td>
<td>75 (62, 85)</td>
</tr>
<tr>
<td>Caucasian, n (%)</td>
<td>22 (100)</td>
</tr>
<tr>
<td>Aetiology of kidney failure</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis, n (%)</td>
<td>10 (45)</td>
</tr>
<tr>
<td>Polycystic kidney disease, n (%)</td>
<td>6 (27)</td>
</tr>
<tr>
<td>Vesicoureteric reflux, n (%)</td>
<td>4 (18)</td>
</tr>
<tr>
<td>Others, n (%)</td>
<td>2 (9)</td>
</tr>
<tr>
<td>Transplant number</td>
<td></td>
</tr>
<tr>
<td>I, n (%)</td>
<td>22 (100)</td>
</tr>
<tr>
<td>Transplant type</td>
<td></td>
</tr>
<tr>
<td>Cadaveric donor, n (%)</td>
<td>17 (77)</td>
</tr>
<tr>
<td>Living donor, n (%)</td>
<td>5 (23)</td>
</tr>
<tr>
<td>Tacrolimus level (BD prednisolone) (ng/mL)</td>
<td>10.5 (8.4, 11.3)</td>
</tr>
<tr>
<td>Tacrolimus level (QD prednisolone) (ng/mL)</td>
<td>10.5 (6.7, 12.5)</td>
</tr>
<tr>
<td>Serum creatinine (μmol/L)</td>
<td>141 (101, 195)</td>
</tr>
</tbody>
</table>

Values are median (IQR).
be important, as glycaemic variability has been associated with adverse outcomes. Both \textit{in vivo} and \textit{in vitro} studies have suggested that glycaemic variability induces oxidative stress to a greater extent than sustained hyperglycaemia, and this may exacerbate chronic β-cell dysfunction [6]. Although not replicated in all studies, glycaemic variability has also been associated with complications of diabetes, including accelerated atherogenesis and microalbuminuria [26–29].

It is believed that most prednisolone effects are mediated through intracellular glucocorticoid receptor binding and genomic signalling [3]. The glucocorticoid receptor–prednisolone complex is localized to the nucleus where dimerization is necessary for interaction with DNA sequences, known as glucocorticoid response elements. Subsequent alterations in gene expression occur slowly (i.e. hours) and are thought to explain some of the metabolic and anti-inflammatory effects of glucocorticoids, including hyperglycaemia [30, 31]. Our data suggest that these glycaemic effects occur predominantly within the initial 12 h after prednisolone dosing and are greater with larger prednisolone doses.

While our study has focused on the reduction in hyperglycaemia obtained with divided dosing of prednisolone, small studies have suggested that this may also enhance immunosuppressive effects [4, 32]. As daily dosing is associated with the absence of measurable drug in the circulation for approximately half of the day, this could reduce its immunosuppressive and anti-inflammatory effects. Both Uhl \textit{et al.} [32] and Czock \textit{et al.} [3] found that divided BD methylprednisolone provided more sustained suppression of CD4+, CD8+ and CD3+ lymphocytes in healthy volunteers. A study by Keller and colleagues highlighted the potential importance of sustained exposure to prednisolone in controlling the alloimmune response. Studying 40 kidney transplant recipients on daily prednisolone, Keller and colleagues observed an association between measured half-life of methylprednisolone and prevention of rejection. In the 18 patients with a rejection episode in the first 6 months post-transplant, they noted a significantly shorter half-life for methylprednisolone compared with the 22 who remained rejection free (2.5 versus 2.9 h, \( P = 0.03 \)) [4]. Further studies are required examining both the therapeutic and adverse effects of divided dosing of prednisolone compared with QD dosing, with a potential outcome being a reduction in total daily dose [5].

\begin{table}[h]
\centering
\caption{Subgroup analyses of glycaemic end points}
\begin{tabular}{|c|c|c|}
\hline
 & QD prednisolone & BD prednisolone & \( P \)-value \\
\hline
Subjects initially commenced on QD prednisolone (\( n = 11 \)) & & & \\
Mean glucose \( (\text{mmol/L/h}) \) & 8.1 ± 2.2 & 8.0 ± 1.7 & 0.946 \\
Peak glucose \( (\text{mmol/L}) \) & 11.9 (10.7, 13.6) & 10.5 (9.8, 12.0) & 0.030 \\
Nadir glucose \( (\text{mmol/L}) \) & 5.4 (4.9, 6.3) & 6.2 (5.6, 7.0) & 0.053 \\
Glucose \( \geq 7.8 \) \( (\text{mmol/L}) \) & 34.9 (33.3, 55.5) & 25.7 (13.1, 34.9) & 0.042 \\
\hline
Subjects initially commenced on BD prednisolone (\( n = 11 \)) & & & \\
Mean glucose \( (\text{mmol/L/h}) \) & 8.1 ± 2.4 & 7.9 ± 1.6 & \(<0.001 \) \\
Peak glucose \( (\text{mmol/L}) \) & 11.4 (10.1, 12.6) & 9.9 (8.5, 11.3) & 0.001 \\
Nadir glucose \( (\text{mmol/L}) \) & 5.8 (4.6, 6.2) & 6.2 (4.7, 6.7) & 0.029 \\
Glucose \( \geq 7.8 \) \( (\text{mmol/L}) \) & 42.6 (32.8, 50.1) & 24.6 (18.6, 28.8) & 0.005 \\
\hline
\end{tabular}
\end{table}

Results are median (IQR) except mean glucose (mean ± SD).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Glycaemic end points: mean glucose (A), peak glucose (B), nadir glucose (C) and exposure to glucose \( \geq 7.8 \text{ mmol/L (} \geq 140 \text{ mg/dL)} \) (D) for QD prednisolone dosing and divided BD prednisolone dosing. Boxes indicate median (IQR) and error bars indicate range.}
\end{figure}
The differing glycaemic profiles demonstrated with the two prednisolone regimens has implications for strategies to screen for hyperglycaemia in kidney transplant recipients receiving prednisolone, and for the selection of hypoglycaemic therapies in these patients. The diurnal impact of prednisolone on glycaemia, with morning nadir and afternoon peak, indicates that...
afternoon blood glucose testing would have greater sensitivity than fasting blood glucose testing for the detection of hyperglycaemia during the early post-transplant period (Figure 4). For patients requiring insulin, the glycaemic effects of daily prednisolone dosing may be best managed with morning administration of intermediate acting insulin (e.g. isophane), while the glycaemic profile associated with BD prednisolone would be best matched with a long-acting insulin, such as glargine or detemir [33–35]. Clearly, specific studies examining the effects of divided dosing in transplant recipients with pre-existing diabetes are required.

Insomnia is frequently reported among patients receiving moderate-to-high dose glucocorticoids, and a single polysomnography study has demonstrated a very small increase in Stage 2 sleep with slight rapid eye movement sleep suppression [36]. There was some concern that taking a portion of prednisolone nocturnally may further aggravate sleep disturbance. Despite this, we found no significant difference in Athens insomnia scale scores, although the study was insufficiently powered to identify small differences.

We have demonstrated the effects of adopting divided prednisolone dosing over a relatively short period. While the results are strictly applicable only to a Caucasian kidney transplant population on a calcineurin inhibitor-based immunosuppression regimen, they have significant implications for the management of inflammatory diseases where higher doses of prednisolone are often employed over a period of many weeks. Interestingly, split dosing of prednisolone has been effective in systemic vasculitis, rheumatoid arthritis, nephrotic syndrome, temporal arteritis and Bell’s palsy [2, 37–39]. While blinding in this study was limited by both clinicians and patients needing to know dosages, bias was minimized by using the blinded-to-patient iPro2 CGM device, and by asking subjects to maintain usual dietary and exercise habits during the 5-day study period, recording these in a diary. Clearly, further studies are needed within and beyond the transplant setting. Future studies examining glucocorticoid-based immunosuppression may also include the evaluation of preparations with a more prolonged action. These may be able to deliver a more sustained immunosuppression, with less glycaemic variability despite infrequent dosing [40].

In conclusion, kidney transplant recipients receiving maintenance prednisolone as part of a calcineurin inhibitor-based regimen experience peak glucose ~7–8 h after morning prednisolone administration, with interstitial fluid glucose levels often ≥11.1 mmol/L (200 mg/dL). This pharmacodynamic pattern suggests that, in patients receiving moderate prednisolone doses, afternoon blood glucose would be a highly sensitive screening test for hyperglycaemia. Dividing the prednisolone into morning and evening doses reduced peak glucose, mean glucose, exposure to hyperglycaemia and glycaemic variability in the early period following kidney transplantation. The magnitude of these differences warrants the performance of further studies in the future.

<table>
<thead>
<tr>
<th>Test</th>
<th>QD prednisolone</th>
<th>BD prednisolone</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard deviation</td>
<td>1.9 (1.6, 2.0)</td>
<td>1.1 (0.8, 1.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAGE</td>
<td>2.2 (1.9, 2.7)</td>
<td>1.5 (1.2, 1.9)</td>
<td>0.001</td>
</tr>
<tr>
<td>CONGA-1</td>
<td>1.2 (0.9, 1.3)</td>
<td>1.0 (0.8, 1.2)</td>
<td>0.039</td>
</tr>
<tr>
<td>CONGA-2</td>
<td>1.4 (1.3, 1.8)</td>
<td>1.2 (1.0, 1.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>J-index</td>
<td>31 (24, 38)</td>
<td>25 (22, 31)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Values are median (IQR).

C.J.Y. was involved in all aspects of this research. S.F., P.G.C. and S.J.C. contributed to study design, data analysis and manuscript writing. C.J.Y. is the guarantor of this manuscript.

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