Abstract

Introduction: Glucocorticoid (GC) therapy deleteriously affects the skeleton including decreasing bone mineral density (BMD) in kidney transplant recipients. BMD improves one year after GC discontinuation and this study aimed to evaluate changes in BMD beyond one year.

Methods: Subjects were previously enrolled one year after kidney transplantation and randomized to continue or stop prednisone. BMD measured by dual-energy X-ray absorptiometry (DXA) was performed at enrollment, 1 year, and repeated at an average of 3.5 years post-randomization. BMD was standardized (sBMD). Models of sBMD were fitted for both groups, adjusting for potential confounders.

Results: At the lumbar spine the slope (g/cm² per year) of sBMD in the withdrawal group increased (0.018, p=0.001) while the continuation group showed no significant change (-0.002, p=0.662), and the difference (0.020, p=0.003) remained significant. At the total hip, slope of sBMD for both groups showed no significant change (p=0.062 and 0.083 for continuation and withdrawal, respectively), but the difference between groups (0.021, p=0.007) was significant. At the femoral neck, no significant changes were seen in the slope of sBMD within or between groups (0.009, p=0.157).

Conclusion: This study provides additional data that GC withdrawal after kidney transplantation leads to increased BMD at the lumbar spine and total hip over a mean 3.5 years follow-up.

ABBREVIATIONS

BMD: Bone Mineral Density; GC: Glucocorticoid; DXA: Dual Absorption X-ray Absorptiometry

INTRODUCTION

Kidney transplantation is the best treatment for end stage renal disease and as overall survival has improved in kidney transplant recipients, clinicians and patient face chronic complications of renal transplantation, such as osteoporosis and fragility fractures [1,2]. Bone mineral density (BMD) rapidly declines during the first year after renal transplant, especially during the first 6 months [3]. After the first year, ongoing bone loss persists [4]. Fracture risk, which is greater in the dialysis versus general population, [5] is further elevated during the first three years after kidney transplantation [6]. Risk factors for post-transplant bone loss in kidney transplant recipients are numerous including preexisting renal osteodystrophy and immune suppressants such as glucocorticoid (GC). Earlier studies indicate that GC pose significant risk for post-transplant osteoporosis in kidney recipients [7].

GC therapy deleteriously affects the skeleton which may be reversible upon its discontinuation [7-10]. A previously published one-year prospective randomized clinical trial showed subjects who withdrew versus those who continued their GC regimen had a significant improvement in their BMD [11]. Our goal was to determine whether the beneficial effect on BMD persisted beyond one year in this cohort.

MATERIALS AND METHODS

Patients were randomized to GC withdrawal or continuation groups. BMD at three sites (lumbar spine, total hip and femoral neck) was measured using Dual Absorption X-ray Absorptiometry.
Baseline characteristics and biochemical data of the 51 subjects (26 GC withdrawal, 25 GC continuation) is shown in (Table 1). No statistical differences could be found between subjects (26 GC withdrawal, 25 GC continuation) is shown in RESULTS AND DISCUSSION using counts and percentages across steroid withdrawal status.

Three individual random-effects linear regression models were developed for each of the three DXA scan locations, lumbar spine, total hip, and femoral neck due to the longitudinal nature of the observations. This method takes into account the between subject and within subject variance and covariance when estimating the standard errors used to test if the coefficients are statistically different from zero. The models included terms for the risk factor, time, the interaction between the risk factor and time, the random term (patient ID), and any confounders. None of the variables were found to be effect modifiers. After running the random-effect linear regression models, linear contrast statements were used to determine the slope (change in sBMD/year) in GC continuation and withdrawal, and the difference in these two slopes. This method uses the Z-statistic when testing the slopes. Additionally, categorical covariates were described using counts and percentages across steroid withdrawal status while continuous covariates were described across groups using means and standard deviations. All analyses were run using Stata 13.1, StataCorp LP, and College Station, TX.

RESULTS AND DISCUSSION

Baseline characteristics and biochemical data of the 51 subjects (26 GC withdrawal, 25 GC continuation) is shown in (Table 1). No statistical differences could be found between the two groups in baseline age, race, gender, body mass index (BMI), type of kidney transplant, time since transplant, diabetes prevalence, duration of hemodialysis or peritoneal dialysis, serum creatinine or creatinine clearance, phosphorus, intact parathyroid hormone (PTH), alkaline phosphatase, cumulative exposure of prednisone, or bone active medications. Baseline standardized BMD or Z-scores at each of the 3 sites also did not differ statistically between the groups. Serum calcium concentration and albumin-corrected calcium were significantly higher in the withdrawal group (p=0.013 and 0.037, respectively).

Using the date of DXA1 as time zero, subjects underwent DXA2 414 ± 91 days after DXA1 (range, 286-941 days) and DXA3 1,307 ± 476 days after DXA1 (range, 717-2,855 days). Values of sBMD at DXA1, DXA2, and DXA3 for each anatomical site are shown in (Figure 1). In the GC withdrawal group, mean lumbar spine sBMD (± SD) at DXA1, DXA2, and DXA3 were 1.033 (± 0.140), 1.086 (± 0.147), and 1.119 (± 0.174), respectively; at the total hip mean 0.955 (± 0.131), 0.990 (± 0.137), and 0.992 (± 0.147), respectively; and femoral neck 0.771 (± 0.120), 0.800 (± 0.129), and 0.826 (± 0.124), respectively. In the GC continuation group, lumbar spine measurements were 1.056 (± 0.168), 1.073 (± 0.167), and 1.067 (± 0.172), respectively; total hip 0.949 (± 0.180), 0.939 (± 0.159), and 0.920 (± 0.173), respectively; and femoral neck 0.765 (± 0.141), 0.800 (± 0.139), and 0.786 (± 0.125), respectively.

Because of the wide range in time between DXA2 and DXA3, we did not test whether sBMD changed between the specific DXA scans. Rather, models of sBMD were fitted over time as single lines, for withdrawal and continuation groups at each anatomical site (Figure 2, left-hand panels). The lumbar spine regression model was adjusted for sex, race, age, BMI, cumulative GC dose, albumin-corrected calcium, alkaline phosphatase, calcitriol use, interaction term between estrogen use and DXA, interaction term between bone-active medications and DXA, phosphorous, and serum creatinine. Total hip was adjusted for sex, race, age, BMI, cumulative GC dose, death, alkaline phosphatase, interaction term between bisphosphonate use and DXA, calcitriol use, diabetes mellitus, interaction term between estrogen and DXA, interaction term between bone-active medications and DXA, phosphorous, and serum creatinine. Femoral neck was adjusted for sex, race, age, BMI, cumulative dose, alkaline phosphatase, interaction term between bisphosphonate and DXA, diabetes mellitus, interaction term between estrogen and DXA, interaction term between bone-active medications and DXA, and serum creatinine. The difference (withdrawal minus continuation) in these fitted lines (Figure 2, right-hand panels), by anatomical site are compared (Table 2).

As shown in Table 2, at the lumbar spine the slope (g/cm² per year) of sBMD in the withdrawal group increased (0.018, p=0.001) while the continuation group showed no significant change (-0.002, p=0.662), and the difference (0.020, p=0.003) remained significant. At the total hip, the slope of sBMD for both groups showed no significant change, but the difference between groups (0.021, p=0.007) was significant. At the femoral neck, no significant changes were seen in the slope of sBMD for either group or in the difference in slopes between groups (0.009, p=0.157). This data suggests that GC withdrawal versus continuation results in a positive impact on BMD at the lumbar spine and total hip and a neutral impact at the femoral neck BMD in the renal transplant patient over time.

Renal transplant patients who discontinue GCs have been...
Table 1: Baseline Characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Overall (SD)</th>
<th>GC Withdrawal (SD) N = 26</th>
<th>GC Continuation (SD) N = 25</th>
<th>p-value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51</td>
<td>51.4 (13.7)</td>
<td>47.8 (15.0)</td>
<td>46.9 (12.5)</td>
<td>0.817</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>51</td>
<td>56.9</td>
<td>53.8</td>
<td>60.0</td>
<td>0.779</td>
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<tr>
<td>Race (% white)</td>
<td>51</td>
<td>76.5</td>
<td>76.9</td>
<td>76.0</td>
<td>&gt; 0.999</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>51</td>
<td>28.9 (4.6)</td>
<td>28.7 (4.8)</td>
<td>29.2 (4.3)</td>
<td>0.697</td>
</tr>
<tr>
<td>GC Withdrawal (SD) N = 26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC Continuation (SD) N = 25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time since transplant (days)</td>
<td>51</td>
<td>383 (199)</td>
<td>387 (183)</td>
<td>378 (218)</td>
<td>0.874</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>51</td>
<td>37.3</td>
<td>28.0</td>
<td>48.0</td>
<td>0.153</td>
</tr>
<tr>
<td>Hemodialysis (months)</td>
<td>51</td>
<td>12.6 (28.6)</td>
<td>11.0 (15.2)</td>
<td>14.3 (38.2)</td>
<td>0.690</td>
</tr>
<tr>
<td>Peritoneal Dialysis (months)</td>
<td>51</td>
<td>7.4 (10.7)</td>
<td>7.4 (9.0)</td>
<td>7.3 (12.5)</td>
<td>0.973</td>
</tr>
<tr>
<td>Calcium (mg/dl)</td>
<td>51</td>
<td>9.5 (0.4)</td>
<td>9.7 (0.4)</td>
<td>9.4 (0.4)</td>
<td>0.013</td>
</tr>
<tr>
<td>Albumin-corrected Calcium (mg/dl)</td>
<td>51</td>
<td>9.6 (0.5)</td>
<td>9.8 (0.7)</td>
<td>9.4 (0.4)</td>
<td>0.037</td>
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<tr>
<td>Phosphorous (mg/dl)</td>
<td>51</td>
<td>2.9 (0.5)</td>
<td>2.9 (0.5)</td>
<td>2.9 (0.6)</td>
<td>0.865</td>
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<tr>
<td>Creatinine (mg/dl)</td>
<td>51</td>
<td>1.48 (0.35)</td>
<td>1.55 (0.35)</td>
<td>1.42 (0.35)</td>
<td>0.212</td>
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<tr>
<td>Creatinine Clearance (ml/min)</td>
<td>51</td>
<td>65.9 (18.3)</td>
<td>63.6 (13.6)</td>
<td>68.3 (22.2)</td>
<td>0.365</td>
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<tr>
<td>Intact PTH (pg/ml)</td>
<td>51</td>
<td>88.8 (61.0)</td>
<td>79.3 (39.4)</td>
<td>97.3 (77.3)</td>
<td>0.297</td>
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<tr>
<td>Alkaline Phosphatase (U/L)</td>
<td>51</td>
<td>95.5 (35.6)</td>
<td>102.5 (38.8)</td>
<td>88.2 (31.0)</td>
<td>0.154</td>
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<tr>
<td>Cumulative Prednisone (mg)</td>
<td>50</td>
<td>7,652 (2,685)</td>
<td>7,648 (2,854)</td>
<td>7,656 (2,564)</td>
<td>0.991</td>
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<tr>
<td>Bone Active Medication (%)</td>
<td>51</td>
<td>23.5</td>
<td>23.1</td>
<td>24.0</td>
<td>&gt; 0.999</td>
</tr>
<tr>
<td>Lumbar Spine sBMD (g/cm²)</td>
<td>50</td>
<td>1.044 (0.154)</td>
<td>1.033 (0.140)</td>
<td>1.056 (0.168)</td>
<td>0.602</td>
</tr>
<tr>
<td>Lumbar Spine Z-score</td>
<td>50</td>
<td>-0.6 (1.3)</td>
<td>-0.6 (1.3)</td>
<td>-0.6 (1.3)</td>
<td>0.982</td>
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<tr>
<td>Femoral Neck sBMD (g/cm²)</td>
<td>49</td>
<td>0.768 (0.129)</td>
<td>0.771 (0.120)</td>
<td>0.765 (0.141)</td>
<td>0.866</td>
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<tr>
<td>Femoral Neck Z-score</td>
<td>48</td>
<td>-0.8 (0.8)</td>
<td>-0.6 (0.8)</td>
<td>-0.9 (0.8)</td>
<td>0.140</td>
</tr>
<tr>
<td>Total Hip sBMD (g/cm²)</td>
<td>48</td>
<td>0.952 (0.155)</td>
<td>0.955 (0.131)</td>
<td>0.949 (0.180)</td>
<td>0.900</td>
</tr>
<tr>
<td>Total Hip Z-score</td>
<td>47</td>
<td>-0.6 (0.9)</td>
<td>-0.6 (0.8)</td>
<td>-0.7 (0.9)</td>
<td>0.456</td>
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</tbody>
</table>

¹p-values based on either a two-sample t-test or Fisher’s exact test

shown to have stable graft function and are not associated with a higher risk of acute rejection or graft loss. In addition, there is improvement of metabolic factors such as cholesterol and glucose, but the positive impact on bone over long periods of time had yet to be confirmed [12-14]. This follow up study examined bone density changes over a mean follow up of 3 ½ years (2-8 years) to extend the previously published one year randomized clinical trial of GC withdrawal vs continuation [11].

Our data shows an improvement in lumbar spine BMD over time and trends of improvement at total hip and femoral neck in the GC withdrawal group. In the GC continuation group, there were trends of declining BMD at all three sites, which is consistent with other studies [4,15]. The annual rate of BMD decrease in the GC continuation group is small and consistent with age-related BMD decline of 0.5-1% per year. To place the data in a clinical context, the mean increases in BMD at 3 years after steroid withdrawal are + 0.054, + 0.030, and 0.021 g/cm² at the lumbar spine, total hip and femoral neck, respectively. The magnitude of improvement between these sites (lumbar spine > total hip > femoral neck) is consistent with the corresponding composition of more metabolically active cancellous bone in the lumbar spine and less metabolically active bone in the femoral neck, with an even admixture in total hip. This extends our previous observation that GC withdrawal had greater effect on more active sites such as trabecular enriched vertebral spine and moderately active sites such as total hip less effect on cortical bone enriched femoral neck [11]. The data is consistent with findings of stable or increased BMD in observational studies of renal transplant recipients on long-term GC therapy followed for more than 10 years [16,17].

The pathophysiology of GC-induced osteoporosis involves multiple complex mechanisms including decreased bone formation, increased bone re sorption, and impaired vitamin D and calcium metabolism [18,19]. The anatomical differential response in bone density to GC withdrawal could explain why different osteoporosis medications have differential efficacy at different anatomical sites [20] and can lead to better selection of anti-osteoporosis medications in individual patients.
Bisphosphonates have been the recommended therapy for GC-induced osteoporosis, but anabolic therapy with teriparatide in selected patients may also improve bone mineral density and decrease fracture rate [21]. However, efficacy of these therapies in the renal transplant patients is uncertain [22]. In addition, steroid withdrawal has been used as an effective therapeutic strategy to decrease well-known GC related metabolic side effects while still being able to maintain good graft function in earlier renal transplant patients [12,23]. The positive impact of GC withdrawal in these studies has yielded a better understanding of the pathophysiology and management of steroid induced osteoporosis.

The primary strength of this study is the longer duration of follow up and ability to demonstrate the sustained positive effect of steroid withdrawal on site-specific BMD. Previously, Farmer et al, reported that stopping glucocorticoid therapy in renal transplant patients 6.5-7.3 years post-transplant lead to a 2.5% increase in lumbar spine BMD 1 year later [10]. Our data reported an RCT but in early glucocorticoid withdrawal at 1 year post-transplant, and found improvements of 4.7% and 2.1% at the lumbar spine and total proximal hip, respectively at 1 year [11]. The current paper presents a longer time of follow up, 3.5 years, and therefore adds to the medical literature in the renal transplant population. Additionally, many relevant potential confounders including bone active agents including bisphosphonates, renal function, PTH were included. The main limitation of this study lies in that fact that a smaller subset of patients had additional DXA scans and at variable time points after completion of the prior randomized trial. Despite this, baseline characteristics between the two groups were not significantly different in factors which influence BMD. A small difference of calcium level was present at baseline between the two groups (9.7 ± 0.4 vs 9.4 ± 0.4 mg/dl), but this was not associated with a significantly different PTH level, suggesting this unlikely contributed to changes in BMD over time. The use of different DXA manufacturers adds imprecision to BMD measurement, but we were able to adopt a previously validated method to standardize the bone density measurements. This study is a reflection of real life practice,

Figure 1 Mean bone mineral density (BMD) by anatomic site, (A) lumbar spine, (B) total hip, and (C) femoral neck. Depicts measured mean standardized BMD (g/cm2) at time of baseline (DXA 1), first follow up (DXA2), and second follow up (DXA3) in both the glucocorticoid withdrawal and continuation groups. Error bars denote standard deviation.

Figure 2 Fitted lines over years after first DXA with the associated 95% confidence intervals (CI) for withdrawal (gray lines) and continuation (black lines) groups are presented. Lines and CIs are based on individual random-effects linear regression models for lumbar spine (panel A), total hip (panel B), and femoral neck (panel C). Panels D, E, and F for lumbar spine, total hip, and femoral neck, respectively show the difference between the two groups (withdrawal minus continuation) over years after first DXA based on the same adjusted models. If the 95% CI does not include zero, then the difference between the two groups is considered statistically significant at the 0.05 level. Lumbar spine (panels A and D) results were adjusted for sex, race, age, BMI, cumulative prednisone dose, albumin-adjusted calcium, alkaline phosphatase, calcitriol use, interaction term between estrogen use and DXA, interaction term between bone-active medication use and DXA, phosphorous, and serum creatinine.Total hip (panels B and E) results were adjusted for sex, race, age, BMI, cumulative prednisone dose, death, alkaline phosphatase, interaction term between bisphosphonate use and DXA, calcitriol use, diabetes mellitus, interaction term between estrogen use and DXA, interaction term between bone-active medication use and DXA, phosphorous, and serum creatinine. Femoral neck (panels C and F) results were adjusted for sex, race, age, BMI, cumulative prednisone dose, alkaline phosphatase, interaction term between bisphosphonate use and DXA, diabetes mellitus, interaction term between estrogen use and DXA, interaction term between bone-active medication use and DXA, and serum creatinine.
which is largely variable in terms of skeletal health management in renal transplant recipients. Some patients had no DXA follow up and some had repeat testing up to 7.8 years later. Effect of additional GC therapy related to transplant rejection on BMD would be expected to decrease BMD and therefore introduce a bias toward a null effect between the two groups. A final limitation on this study is the lack of fracture data.

Although BMD testing is useful to predict fracture in the general population, and is lower in predialysis patients with fracture versus no fracture, [24] its predictive ability in CKD5D is uncertain [25]. Thus, guidelines suggest BMD testing not be performed routinely in CKD stage 3-5D, but do suggest obtaining DXA scan in the first 3 months following kidney transplant in those with estimated glomerular filtration rate >30 mL/min/1.73 m² [26]. The latter guideline was offered with the weakest grade evidence and currently undergoing revision [27] taking into account recent data suggesting BMD classification between normal, osteopenia, and osteoporosis predicts future fracture. [16] In the current era of early GC withdrawal after transplantation, BMD does not change at the spine or hip but decreased at the forearm at 1 year by DXA scanning and use of high-resolution peripheral quantitative CT scanning (which separates cortical from cancellous bone compartments in 3 dimension), parameters of bone strength worsened: trabecular density, cortical area, thickness, density, porosity at the distal radius and tibia at 1 year which was associated with higher PTH levels [28,29]. The clinical utility of BMD measurement in the renal transplant population remains to be elucidated.

**CONCLUSION**

In conclusion, this study provides additional data that glucocorticoid withdrawal versus continuation in renal transplant recipients leads to increasing BMD at the lumbar spine and total hip at an average of 3 ½ year follow up.

**ACKNOWLEDGEMENTS**

We thank Drs. Todd Pesavento, Elizabeth Davies, Uday Nori for their clinical care of patients who participated in this trial.

**REFERENCES**


