



HOKKAIDO UNIVERSITY

Title	Changes in bone turnover markers after discontinuing long-term glucocorticoid administration in children with idiopathic nephrotic syndrome : a multicenter retrospective observational study
Author(s)	Ueda, Yasuhiro; Okamoto, Takayuki; Sato, Yasuyuki et al.
Citation	Pediatric nephrology, 38(10), 3285-3296 https://doi.org/10.1007/s00467-023-05966-2
Issue Date	2023-10-01
Doc URL	https://hdl.handle.net/2115/93497
Rights	This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature' s AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any corrections. The Version of Record is available online at: http://dx.doi.org/10.1007/s00467-023-05966-2 .
Type	journal article
File Information	Pediatr Nephrol_s00467-023-05966-2.pdf



Title:

Changes in bone turnover markers after discontinuing long-term glucocorticoid administration in children with idiopathic nephrotic syndrome: A multicenter retrospective observational study

Author information

Yasuhiro Ueda¹, Takayuki Okamoto¹, Yasuyuki Sato¹, Asako Hayashi¹,
Toshiyuki Takahashi¹, Ryota Suzuki¹, Hayato Aoyagi², Michihiko Ueno³, Norio
Kobayashi⁴, Kimiaki Uetake⁵, Masanori Nakanishi⁶, Tadashi Ariga^{1,3}, Atsushi
Manabe¹

Affiliation

¹Department of Pediatrics, Hokkaido University Graduate School of Medicine,
Sapporo, Hokkaido, Japan

²Department of Pediatrics, Obihiro Kyokai Hospital, Obihiro, Hokkaido, Japan

³Department of Pediatrics, Nikko Memorial Hospital, Muroran, Hokkaido, Japan

⁴Department of Pediatrics, Oji General Hospital, Tomakomai, Hokkaido, Japan

⁵Department of Pediatrics, Obihiro Kosei Hospital, Obihiro, Hokkaido, Japan

⁶Department of Pediatrics, Kushiro Red Cross Hospital, Kushiro, Hokkaido,
Japan

Corresponding author: Takayuki Okamoto, MD, PhD

Department of Pediatrics, Hokkaido University Graduate School of Medicine,
North 15, West 7, Sapporo, Japan

Email address: okamon@med.hokudai.ac.jp

ORCID

Yasuhiro Ueda: 0000-0003-4205-320X

Takayuki Okamoto: 0000-0002-6029-451X

Yasuyuki Sato: 0000-0002-7804-5490

Asako Hayashi: 0000-0002-9023-0904

Toshiyuki Takahashi: 0000-0002-8783-3528

Ryota Suzuki: 0000-0002-3021-8348

Michihiko Ueno: 0000-0002-6542-3173

Atsushi Manabe: 0000-0002-6698-2348

ABSTRACT

Background

Glucocorticoids affect bone turnover. Little is known about how bone turnover changes when glucocorticoids are discontinued following long-term administration.

Methods

This retrospective observational study was conducted on the relationship between discontinuation of long-term administration of glucocorticoid and bone turnover markers (BTMs) in patients with childhood-onset idiopathic nephrotic syndrome. Serum bone alkaline phosphatase (BAP), intact procollagen type 1 N-terminal propeptide (P1NP), and tartrate-resistant acid phosphatase-5b (TRACP-5b) were evaluated as BTMs.

Results

Thirty-eight pairs of BTMs at glucocorticoid administration and after discontinuation were analyzed in 29 patients. The median age at baseline was 12.4 (interquartile range, 9.0–14.5) years, and the median time from the onset of nephrotic syndrome was 5.9 (3.3–9.7) years. The mean period from prednisolone discontinuation to the measurement of BTMs after glucocorticoid

discontinuation was 3.5 ± 1.0 months. Changes in BTMs after glucocorticoid discontinuation were modest when the daily prednisolone dose was <0.25 mg/kg/day (ln BAP standard deviation [SD] score, $p=0.19$; log intact P1NP SD score, $p=0.70$; TRACP-5b, $p=0.95$). When the daily prednisolone dose was ≥ 0.25 mg/kg/day, all BTMs increased significantly after glucocorticoid discontinuation (ln BAP SD score, $p<0.01$; log intact P1NP SD score, $p<0.01$; TRACP-5b, $p<0.01$).

Conclusions

Decreased BTMs can rise within a few months of discontinuing long-term glucocorticoid administration. When the administered glucocorticoid dose is low, changes in BTMs may be small.

Keywords

Bone turnover markers, Children, Glucocorticoid, Nephrotic syndrome, Rituximab

1 INTRODUCTION

2 Glucocorticoid (GC) has adverse effects on the bone through various
3 mechanisms, and long-term administration of GC causes osteoporosis, called
4 GC-induced osteoporosis [1]. GC inhibits bone formation by suppressing
5 osteoblastogenesis and promoting osteoblast apoptosis [2]. Although there are
6 various reports on the effects of GC on bone resorption with some inconsistent
7 results [1, 3], GC stimulates bone resorption, at least in the early stage of GC
8 administration, promotes osteoclastogenesis and maturation of osteoclasts, and
9 prolongs osteoclast survival [1, 4].

10 At the consensus conference in 2000 by the National Institute of Health,
11 osteoporosis was defined as “a skeletal disorder characterized by compromised
12 bone strength predisposing a person to an increased risk of fracture [5].” Bone
13 strength is determined by bone density and bone quality [5]. Therefore, it is
14 important to evaluate bone health from static and dynamic aspects [6]. The
15 former representatives are bone mineral density (BMD) measurement by dual-
16 energy X-ray absorptiometry (DXA) or peripheral quantitative computed
17 tomography, and the latter representatives are bone biopsy or measurement of
18 bone turnover markers (BTMs). BTMs are used to monitor treatment response

1 and, to a certain degree, predict fractures in osteoporosis treatment in adults
2 [7]. BTMs are divided into bone formation and resorption markers [6]. Bone
3 formation markers are enzymes of osteoblasts or substances produced during
4 collagen formation that reflect the activity of osteoblasts. Bone resorption
5 markers are enzymes of osteoclasts or collagen degradation products, which
6 reflect the activity of osteoclasts.

7 Many studies on changes in BTMs shortly after initiating GC
8 administration have reported that bone formation markers decreased in most
9 cases [8–15]. Studies on changes in bone resorption markers after the initiation
10 of GC are conflicting, with many reports indicating an increase in bone
11 resorption markers in adults and a decrease in children [8–11, 13, 14, 16]. The
12 reason for this difference is poorly understood.

13 There are few reports on changes in BTMs after the discontinuation of
14 GC. Previous reports have suggested that if the duration of GC administration is
15 relatively short, changed BTMs appear to recover rapidly after discontinuation of
16 GC [13, 17–19]. However, changes in BTMs after the discontinuation of long-
17 term GC administration have not been well described.

1 In recent years, as rituximab has been administered to maintain
2 remission of nephrotic syndrome (NS), GC administration can be stopped, at
3 least temporarily, even in patients forced to continue long-term GC
4 administration [20, 21]. The main objective of the present study was to
5 investigate changes in BTMs after discontinuation of long-term GC
6 administration in patients with childhood-onset idiopathic NS who could
7 discontinue GC after rituximab administration.

8

METHODS

Study design and participants

This retrospective observational study was conducted on Japanese patients with childhood-onset idiopathic NS who were admitted and received the first administration of rituximab at a tertiary medical center in Japan between January 2015 and March 2021. These patients had attended the tertiary center or were referred to the center from other hospitals in Hokkaido, Japan, due to the refractory course of NS and possible indications for rituximab administration.

In almost all the patients who were administered rituximab, BTMs were measured at every rituximab administration as a routine practice. Some of the subjects in the present study were participants of a previously performed clinical study to assess the usefulness of rituximab in pediatric NS [22]. In addition, for a part of the subjects in the present study, the effects of rituximab administration on glucose tolerance or quality of life have already been reported elsewhere [23, 24]. The plan of the present study was posted on the hospital website, and the subjects could refuse to be included by the opt-out method. The ethical review board of Hokkaido University Hospital approved this study (study number: 021-0094).

1 Patient information and data were extracted from the electronic medical
2 records. Patients were eligible when the following conditions were met: 1) <18
3 years old at the first administration of rituximab, 2) received at least two doses
4 of rituximab, and 3) BTMs were measured each time of rituximab administration
5 and BTMs at GC administration and after GC discontinuation could be
6 consecutively evaluated. Data <18 years of age were included for each patient,
7 and those ≥ 18 years of age were excluded from the analysis. Patients were
8 excluded when they had fractures within a year before the BTMs measurement
9 to be analyzed, had disorders that can influence bone turnover, or took
10 medications that can affect bone turnover. The period “a year” from fracture to
11 BTMs measurement was determined based on reports that some BTMs may
12 take up to a year after fracture to return to baseline levels [25]. Although
13 administration of vitamin D may affect bone turnover [26], more than half of the
14 subjects in the present study were taking an activated vitamin D₃ analog, and
15 these patients were included. Although it is not known how the nephrotic state
16 affects BTMs, serum BTMs measured in the presence of proteinuria were also
17 included in the analysis.

Definitions

Childhood-onset idiopathic NS was defined as NS with an onset before the age of 18 years and with no apparent causative or systemic disorder. NS was diagnosed by a first-morning urine protein to creatinine ratio ≥ 2.0 g/g creatinine and serum albumin < 2.5 g/dL [27]. Remission was defined as a first-morning urinary protein negative or trace by urine dipstick for three consecutive days [27]. Relapse was defined as a first-morning urinary protein $\geq 3+$ on a urine dipstick for three consecutive days [27]. Frequently relapsing NS (FRNS) was defined as having $\geq$ two relapses within six months after the first remission or $\geq$ four relapses within 12 months [27]. Steroid-dependent NS (SDNS) was defined as two consecutive relapses during GC tapering or within two weeks after the discontinuation of GC [27]. Steroid-resistant NS (SRNS) was defined as the absence of remission after daily GC administration for four weeks [28].

GC administration and rituximab

The GC administration method from the onset of NS to the first administration of rituximab in each patient was at the discretion of the treating physicians. Rituximab was administered every four to six months, and the total

number of administrations of rituximab varied from 2 to 10, depending on the patient's condition. To prevent infusion reaction, intravenous methylprednisolone 1–1.5 mg/kg (125 mg for patients with a body weight of ≥ 50 kg or ≥ 16 years old), oral acetaminophen, and oral *d*-chlorpheniramine maleate were administered 30 minutes before rituximab administration.

After introducing rituximab, GC was tapered off as following method based on our established protocol (Fig. 1): When prednisolone (PSL) was administered daily at the time of rituximab administration, tapering was initiated after two weeks. The daily dose remained unchanged and was administered on alternate days from two weeks after rituximab administration (e.g., from 60 mg/day to 60 mg on alternate days), then halved every two weeks, and discontinued (e.g., 60 mg on alternate days, two weeks; 30 mg on alternate days, two weeks; 15 mg on alternate days, two weeks; 7.5 mg on alternate days, two weeks; off). When PSL was administered on alternate days at the time of rituximab administration, the dose was halved from two weeks after rituximab administration on alternate days and then halved every two weeks after. In case of relapse after the introduction of rituximab, GC administration was performed as follows. PSL 60 mg/m² or 2 mg/kg (maximum 60 mg) was

administered daily until remission and was tapered as follows: 60 mg/m² or 2 mg/kg (maximum 60 mg) on alternate days, two weeks; 30 mg/m² or 1 mg/kg (maximum 30 mg) on alternate days, two weeks; 15 mg/m² or 0.5 mg/kg (maximum 15 mg) on alternate days, two weeks; 7.5 mg/m² or 0.25 mg/kg (maximum 7.5 mg) on alternate days, two weeks; off. Regarding the PSL administration after the introduction of rituximab, there were minor modifications at the treating physicians' discretion.

Data collection and analysis

The first time point of measurement of BTMs at GC administration to be analyzed in each patient was defined as "baseline." The first administration of rituximab in each patient was not necessarily the baseline. The extracted baseline data included sex, histopathologic type, history of SRNS, number of relapses, age at onset of NS, age at baseline, duration of NS, administration of immunosuppressants, height, weight, body mass index (BMI), laboratory data (BTMs, serum albumin, creatinine, calcium, phosphate, intact parathyroid hormone (PTH)), lumbar BMD, the presence or absence of activated vitamin D₃ analog administration, and information on PSL administration. For height,

weight, and BMI (weight in kilograms divided by height in meters squared), standard deviation (SD) scores were calculated using standard data from Japanese children [29, 30].

As a routine practice, before each rituximab administration (on the day of administration or within a few days before), non-fasting blood was collected between 10 am and 12 am. The measured BTMs were serum bone alkaline phosphatase (BAP) and serum intact procollagen type 1 N-terminal propeptide (P1NP) as bone formation markers and serum tartrate-resistant acid phosphatase-5b (TRACP-5b), as a bone resorption marker. The estimated glomerular filtration rate was calculated using the creatinine-based Japanese pediatric formula [31]. When the serum albumin concentration was <4 g/dL, the serum calcium concentration was corrected using the following formula:

$$\text{adjusted calcium concentration (mg/dL)} = \text{measured calcium concentration (mg/dL)} + (4 - \text{albumin concentration (g/dL)})$$

Lumbar BMD was measured by DXA using Hologic Discovery-A (Hologic, Inc., Marlborough, MA, USA). BMD was measured from the first to the fourth lumbar vertebrae in children <6 years old and from the second to the fourth lumbar vertebrae in children ≥6 years old, respectively, according to manufacturer's instruction. Calibration was performed

1 daily using a lumbar phantom. BMD values were converted to Z-scores based
2 on the manufacturer-provided reference values for age and sex in Japanese
3 children.

4 The daily PSL dose at the time of BTM measurement at GC
5 administration was converted to the PSL dose per body weight (mg/kg/day).
6 When PSL was administered on alternate days, the value divided by two was
7 considered the daily PSL dose (e.g., when PSL 30 mg was administered on
8 alternate days, the daily PSL dose was 15 mg/day). BTMs at GC administration
9 and consecutive BTMs after GC discontinuation were set as a "pair" (Fig. 1),
10 and changes in BTMs were assessed. When appropriate, the analysis of
11 multiple pairs of BTMs in the same patient was allowed.

13 **Biochemical analysis**

14 For samples for BTM measurement, the blood was centrifuged within an
15 hour after blood collection, and the serum was stored at -80°C until analysis.

16 The BTMs were measured according to the manufacturer's instructions. Serum
17 BAP was measured using a chemiluminescent enzyme immunoassay (Access
18 Ostase, Beckman Coulter, Brea, CA, USA); the intra-assay coefficient of

variation (CV) was 1.5–2.6%. Serum intact P1NP was measured using a radioimmunoassay (Procollagen Intact PINP, FUJI-REBIO, Tokyo, Japan); the intra-assay CV was $\leq 15\%$. Serum TRACP-5b levels were measured using an enzyme-linked immunosorbent assay (Osteolinks TRAP-5b, SB Bioscience, Tokyo, Japan); the intra-assay CV was $\leq 15\%$. For BAP and intact P1NP, SD scores were calculated after natural logarithmic and logarithmic transformation, respectively, using age- and sex-specific reference data in European children [32, 33] because it has been reported that BAP and intact P1NP are not normally distributed by age. For TRACP-5b, the measured values were used because no significant changes in puberty were reported [32].

Primary outcome

The relationship between daily PSL dose at GC administration and changes in BTMs after GC discontinuation was investigated.

Statistical analysis

Missing data were excluded from the analysis. The normality of data was assessed using the Shapiro-Wilk test. Categorical variables were

presented as frequencies and proportions. Continuous variables were expressed as mean and SD or median and interquartile range, according to the distribution of the data. Continuous variables between two groups were analyzed using the Student's *t*-test, the paired *t*-test or Wilcoxon signed-rank test, as appropriate. Correlations between two variables were assessed using Pearson's correlation coefficient. *P* value <0.05 was considered statistically significant. Statistical analyses were performed using JMP Pro 16.2.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Of the 49 patients who received their first dose of rituximab between January 2015 and March 2021, 37 were eligible (Fig. 2). Eight patients were excluded due to bisphosphonate treatment history, endocrine disorders, or recent fractures. Finally, 29 patients were analyzed, a total of 38 pairs of BTMs at GC administration and after GC discontinuation. The analyzed pairs of BTMs included one pair in 21 patients, two in 7 patients, and three in 1 patient.

The baseline clinical characteristics of the 29 patients are presented in Table 1. Female patients were 11 (38%), and males 18 (62%). Seven (24%)

1 patients had a history of SRNS. The median age of onset of NS was 3.8 (2.6–
2 6.6) years old, the median age at baseline was 12.4 (9.0–14.5) years old, and
3 the median duration of NS was 5.9 (3.3–9.7) years. Due to a lack of detailed
4 data, the actual duration of GC administration was determined in only 13
5 patients (44%), which was 4.4 ± 3.0 years. Mean SD scores for height, weight,
6 and BMI at baseline were -1.26 ± 1.26 , -0.25 ± 1.24 , and 0.43 ± 1.19 , respectively
7 ($n = 28$ due to missing height data for a patient). Twenty (69%) patients were
8 administered an activated vitamin D₃ analog at baseline.

9 The characteristics of the 38 pairs of BTMs measured at GC
10 administration and after GC discontinuation are shown in Table 2. The median
11 time from the most recent GC dose increase due to relapse to the measurement
12 of BTMs at GC administration was 40 (17–118) days. The median daily PSL
13 dose at GC administration was 17.5 (4.7–31.3) mg/day, 0.44 (0.11–0.98)
14 mg/kg/day. PSL was administered daily in 16 pairs (42%) and on alternate days
15 in 22 pairs (58%) at GC administration. The mean period from discontinuation of
16 PSL to the measurement of BTMs after discontinuation of GC was 3.5 ± 1.0
17 months. The median age at GC administration and after GC discontinuation
18 was 12.8 (9.7–14.6) and 13.3 (10.2–15.1) years old, respectively. The height SD

score was higher, and the weight and BMI SD scores were lower after GC discontinuation than at GC administration. The serum albumin levels were higher after GC discontinuation than at GC administration. Serum phosphate levels were higher after GC discontinuation. Although serum intact PTH levels were lower after GC discontinuation, the levels remained within the normal range.

Changes in BTMs between GC administration and discontinuation are shown in Table 3. All BTMs were significantly higher after GC discontinuation than at GC administration (BAP:56.1 (31.0–74.5) $\mu\text{g/L}$ vs. 96.4 (57.4–109.6) $\mu\text{g/L}$, $p<0.01$; intact P1NP:170.6 (97.1–415.1) $\mu\text{g/L}$ vs. 476.3 (314.7–703.2) $\mu\text{g/L}$, $p<0.01$; TRACP-5b:16.1 $\pm$ 5.6 U/L vs. 20.8 $\pm$ 8.2 U/L, $p<0.01$). For BAP and intact P1NP, these differences were significant even after conversion to SD scores (ln BAP SD score: 0.06 (-0.71–0.93) vs. 1.43 (0.89–1.79), $p<0.01$; log intact P1NP SD score: -2.78 (-4.36–2.74) vs. 0.11 (-0.58–1.22), $p<0.01$).

Correlations between changes in factors that can affect bone turnover and changes in BTMs after GC discontinuation are shown in Table 4. The daily PSL dose (mg/kg/day) showed significant positive correlations with changes in all BTMs. In contrast, changes in height SD score, serum albumin, phosphate,

1 and intact PTH levels after GC discontinuation did not significantly correlate with
2 changes in any BTMs.

3 To investigate the effect of daily PSL dose at GC administration on
4 changes in BTMs after GC discontinuation, we assessed the changes in BTMs
5 after GC discontinuation by categorizing them into the following four groups
6 according to daily PSL dose at GC administration: PSL <0.25 mg/kg/day, 0.25–
7 0.49 mg/kg/day, 0.50–0.99 mg/kg/day, and ≥1.0 mg/kg/day (Fig. 3). Because
8 the thresholds of daily PSL dose affecting BTMs are unknown, these cutoff
9 values were determined based on the method of PSL tapering at the relapse of
10 NS in children (i.e., modified International Study of Kidney Disease in Children
11 regimen in the Clinical practice guideline for pediatric idiopathic nephrotic
12 syndrome in Japan [34]). Changes in BTMs tended to be mild when the daily
13 PSL dose at GC administration was <0.25 mg/kg/day, and BTMs tended to be
14 elevated in the other three groups.

15 Based on this result, changes in BTMs after GC discontinuation were
16 analyzed in two groups according to daily PSL dose with a cutoff of 0.25
17 mg/kg/day (Fig. 4). The median daily PSL dose at GC administration was 0.08
18 (0.07–0.14) mg/kg/day in the group with a daily PSL dose <0.25 mg/kg/day and

0.90 (0.47–1.19) mg/kg/day in the group with a daily PSL dose ≥ 0.25 mg/kg/day, respectively. In the group with a daily PSL dose < 0.25 mg/kg/day (14 pairs), PSL was administered daily in 1 pair and on alternate days in 13 pairs. In the group with a daily PSL dose ≥ 0.25 mg/kg/day (24 pairs), PSL was administered daily in 15 pairs and on alternate days in 9 pairs. Changes in BTMs were modest when the daily PSL dose was < 0.25 mg/kg/day (ln BAP SD score: 0.68 (-0.00–1.74) vs. 1.32 (0.38–1.86), $p=0.19$; log intact P1NP SD score: -0.34 (-1.15–1.07) vs. 0.03 (-0.78–0.50), $p=0.70$; TRACP-5b: 18.5 (13.6–21.5) U/L vs. 16.9 (13.4–25.1) U/L, $p=0.95$). However, when the daily PSL dose was ≥ 0.25 mg/kg/day, BTMs after GC discontinuation were significantly higher than those at GC administration (ln BAP SD score: -0.45 (-1.32–0.38) vs. 1.42 (1.30–1.75), $p<0.01$; log intact P1NP SD score: -3.81 (-4.92–-2.75) vs. 0.56 (-0.47–1.41), $p<0.01$; TRACP-5b: 13.6 (11.1–18.1) U/L vs. 21.6 (14.0–27.7) U/L, $p<0.01$). In addition, for the BTMs at GC administration ('pre' in Fig. 4), all BTMs tended to be in the normal range in the group with a daily PSL dose < 0.25 mg/kg/day, while those in the group with a daily PSL dose ≥ 0.25 mg/kg/day were lower (ln BAP SD score: 0.68 (-0.00–1.74) vs. -0.45 (-1.32–0.38), $p<0.01$;

1 log intact P1NP SD score: -0.34 (-1.15–1.07) vs. -3.81 (-4.92–2.75), $p<0.01$;

2 TRACP-5b: 18.5 (13.6–21.5) U/L vs. 13.6 (11.1–18.1) U/L, $p<0.05$).

3 In the group with a daily PSL dose <0.25 mg/kg/day, both lumbar BMD

4 and BMD Z-score increased significantly after GC discontinuation (lumbar BMD:

5 0.62 (0.56 – 0.76) g/cm² vs. 0.69 (0.61 – 0.80) g/cm² ($p<0.01$); lumbar BMD Z-

6 score: -2.1 (-3.7 – -1.1) vs. -1.8 (-3.2 – -0.9) ($p<0.05$). In the group with a daily

7 PSL dose ≥ 0.25 mg/kg/day, lumbar BMD increased significantly, while BMD Z-

8 score also increased. However, the latter did not reach statistical significance

9 (lumbar BMD: 0.61 (0.53 – 0.77) g/cm² vs. 0.68 (0.54 – 0.81) g/cm² ($p<0.01$);

10 lumbar BMD Z-score -2.1 (-3.2 – -1.3) vs. -1.9 (-2.8 – -0.5) ($p=0.09$).

12 DISCUSSION

13 The present study suggests that decreased BTMs can rise within a few

14 months after discontinuing long-term GC administration. In addition, when the

15 administered GC dose is low, changes in BTMs may be small.

16 The present study showed that BTMs, which decreased due to long-

17 term GC administration for several years, could increase after discontinuation of

18 GC in children with idiopathic NS. Other than GC, various factors affect bone

turnover, including age, sex, growth, puberty, exercise, and diet [7]. Moreover, BTMs are difficult to interpret in rapidly growing children [6]. In the present study, increases in height SD scores were observed after GC discontinuation, reflecting an increase in growth rate after GC cessation. In addition, when measuring BTMs at GC administration, serum albumin levels were low, reflecting the difficulty in controlling NS relapse before rituximab administration. Serum phosphorus levels were higher, and serum intact PTH levels were lower after GC discontinuation than at GC administration, possibly related to relapse. These factors may have contributed to changes in BTMs. However, while the daily PSL dose at GC administration showed a significant correlation with changes in BTMs, changes in height SD score, levels of serum albumin, serum phosphorus and serum intact PTH did not significantly correlate with changes in BTMs, suggesting that GC discontinuation was the main contributor to changes in BTMs.

The reversibility of BTMs after discontinuing long-term GC administration observed in the present study is consistent with reports that osteocalcin, a bone formation marker, increased within days to weeks after surgery in patients with Cushing's syndrome, a condition in which exposure to

1 endogenous GC exists [35, 36]. Future studies are needed to determine
2 whether the reversal of decreased BTMs is related to clinical outcomes such as
3 future BMD or fracture risk. On the other hand, in a retrospective study in Italy,
4 results contrary to ours have been reported on the relationship between GC and
5 a bone formation marker [15]. Osteocalcin, a bone formation marker, at the time
6 of GC initiation, GC discontinuation (2.44 ± 0.75 years after GC initiation), and a
7 year after GC discontinuation were assessed in pediatric patients with NS. The
8 levels of osteocalcin at the time of GC discontinuation were higher than those at
9 GC initiation and then decreased a year after GC discontinuation. In this study,
10 the number of relapses was low (2.86 ± 0.83), and it may be possible that the GC
11 dose could be reduced to a low level at the time of GC discontinuation (although
12 the exact daily GC dose at the measurement of osteocalcin was not mentioned
13 in the paper), or that change in osteocalcin at measurement was influenced by
14 the prepubertal state.

15 It was suggested that when the administered GC dose is low, changes
16 in BTMs may be small. In the present study, when the daily PSL dose was
17 <0.25 mg/kg/day, all BTMs at GC administration tended to be in the normal
18 range. In the group with a daily PSL dose <0.25 mg/kg/day, PSL was

administered on alternate days in most pairs, which may have contributed to the mild changes in BTMs. A study from Turkey that assessed osteocalcin levels before initiating GC and after 12 weeks of GC administration in 20 pediatric NS patients (4 at initial onset and 16 at relapse) found that osteocalcin decreased after 12 weeks of GC administration [12]. In this study, osteocalcin levels in patients with FRNS decreased significantly compared to those in infrequent relapsers. The study concluded that the greater the cumulative dose of GC, the greater the change in osteocalcin. Although the daily GC dose at the time of osteocalcin measurement is not described, the results are similar to the present study in that the higher the GC dose, the stronger the effect on BTMs.

Regarding BMD, lumbar BMD and BMD Z-score increased after GC discontinuation in each group in the present study. However, the lumbar BMD Z-score in the group with a daily PSL dose <0.25 mg/kg/day increased significantly, while the increase in BMD Z-score in the group with a daily PSL dose ≥ 0.25 mg/kg/day did not reach statistical significance. It is possible that lower GC dose and smaller decrease in BTMs might be associated with improvement in BMD. However, no definitive conclusion can be drawn from the present study. It should be noted that the PSL cutoff value of 0.25 mg/kg/day in

the present study was derived from the analysis process in this study and does not represent the threshold or safety margin for adverse effects of GC on bone health. A prospective study of 100 pediatric NS patients in India evaluated BMD after long-term GC administration [37, 38]. BMD at 3.2 ± 2.7 years after NS onset were lower in patients with FRNS, SDNS, and SRNS, who had taken higher GC doses, than those with infrequent relapses. The report concluded that a greater cumulative GC dose is a risk factor for GC-induced osteoporosis. On the other hand, even small doses of GC (average 71 $\mu\text{g/day}$ as PSL) have been reported to increase the risk of fracture in adults [39], suggesting that lower GC dose may not necessarily prevent fractures. In addition, it was reported that, in postmenopausal women, even administration of 5 mg/day of PSL decreased bone formation markers [40]. The responsiveness of BTMs to GC may differ between children and adults.

In adults, BTMs are clinically applied to monitor the response to treatment for osteoporosis and, to some extent, may be useful in predicting fracture risk [7]. Because GC-induced osteoporosis is rarely treated aggressively in children, unlike in adults, and because it is difficult to evaluate BTMs due to the effects of growth and puberty on bone turnover [6, 41], the

1 clinical application of BTMs has not progressed in children. However, in recent
2 years, there have been increasing reports on the usefulness and safety of
3 bisphosphonates, which are off-label in most countries, as a treatment for
4 pediatric GC-induced osteoporosis [42] and may become an option for future
5 treatment. In addition, with the advent of new anti-osteoporosis drugs, such as
6 anti-receptor activator of nuclear factor-kappa B ligand antibody, recombinant
7 human PTH, or anti-sclerostin antibody [42], BTMs may be used to monitor
8 therapeutic effects in the clinical trial stage or actual clinical settings in children
9 in the future.

10 This study has several limitations. First, it was a retrospective study;
11 hence, we could not evaluate the pubertal stage, calcium and vitamin D intake,
12 and serum 25-hydroxyvitamin D levels, which can affect bone turnover. Second,
13 regarding PSL dose, only the daily PSL dose at the time of BTM measurement
14 was evaluated, and the cumulative PSL dose or average PSL dose over a
15 certain period could not be evaluated. Third, BTMs were standardized using
16 European pediatric data [32, 33] because there is no reference data on BTMs in
17 Japanese children.

1 A strength of the present study is that it assessed BTMs in patients with
2 NS. Although cytokine elevations have been reported in NS [43], it is considered
3 an easy-to-use model for the effects of GC on bone rather than rheumatic
4 disease or inflammatory bowel disease due to the absence of significant
5 systemic inflammation [44]. Because the present study is retrospective and
6 lacks data, we could not evaluate BTMs before GC initiation and cumulative GC
7 dose, and there could be confounding factors for GC dose and changes in
8 BTMs, such as immunosuppressants other than GC and puberty. In the future,
9 prospective studies on changes in BTMs, including data before initiation or dose
10 escalation of GC due to relapse in patients with SDNS or FRNS regardless of
11 rituximab administration, may clarify the association between GC and changes
12 in BTMs.

13 In conclusion, decreased BTMs can rise within a few months after
14 discontinuing long-term GC administration. When the administered GC dose is
15 low, changes in BTMs may be small.

17 **Research involving Human Participants and/or Animals**

18 **Ethics approval**

The study was approved by the ethics committee of Hokkaido University Hospital (study number: 021-0094). This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate

Because the present study was a retrospective study using medical record, informed consent was not required. The subjects could refuse to be included in the study by the opt-out method on the website of Hokkaido University Hospital.

Authors' contribution

Conceptualization: YU, TO, and TT, — Data acquisition: YU, TO, TT, HA, MU, NK, KU, MN, and TA; Writing – original draft preparation: YU; Writing – review and editing: TO, YS, AH, TT, RS, and AM.

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing.

Statements and Declarations

Funding

The authors did not receive support from any organization for the submitted work.

1 **Conflicts of Interest**

2 The authors have no relevant financial or non-financial interests to disclose.

3

4 A part of the present study was presented at the 19th Congress of the

5 International Pediatric Nephrology Association, Calgary, Canada, 2022.

6

7

1 REFERENCES

- 2 1. Chotiarnwong P, McCloskey EV (2020) Pathogenesis of glucocorticoid-
3 induced osteoporosis and options for treatment. *Nat Rev Endocrinol*
4 16:437–447. <https://doi.org/10.1038/s41574-020-0341-0>
- 5 2. Weinstein RS, Jilka RL, Parfitt AM, Manolagas SC (1998) Inhibition of
6 osteoblastogenesis and promotion of apoptosis of osteoblasts and
7 osteocytes by glucocorticoids. Potential mechanisms of their deleterious
8 effects on bone. *J Clin Invest* 102:274–282.
9 <https://doi.org/10.1172/JCI2799>
- 10 3. Bornefalk E, Dahlén I, Michaëlsson K, Ljunggren Ö, Ljunghall S (1998)
11 Age-dependent effect of oral glucocorticoids on markers of bone
12 resorption in patients with acute asthma. *Calcif Tissue Int* 63:9–13.
13 <https://doi.org/10.1007/s002239900482>
- 14 4. Compston J (2018) Glucocorticoid-induced osteoporosis: an update.
15 *Endocrine* 61:7–16. <https://doi.org/10.1007/s12020-018-1588-2>
- 16 5. NIH Consensus Development Panel on Osteoporosis Prevention,
17 Diagnosis, and Therapy (2001) Osteoporosis prevention, diagnosis, and
18 therapy. *JAMA* 285:785–795. <https://doi.org/10.1001/jama.285.6.785>

- 1 6. Jürimäe J (2010) Interpretation and application of bone turnover markers
2 in children and adolescents. *Curr Opin Pediatr* 22:494–500.
3 <https://doi.org/10.1097/MOP.0b013e32833b0b9e>
- 4 7. Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A,
5 McClung M, Morris HA, Silverman S, Trenti T, Wahl DA, Cooper C, Kanis
6 JA; IOF-IFCC Bone Marker Standards Working Group (2011) Markers of
7 bone turnover for the prediction of fracture risk and monitoring of
8 osteoporosis treatment: a need for international reference standards.
9 *Osteoporos Int* 22:391–420. <https://doi.org/10.1007/s00198-010-1501-1>
- 10 8. Wolthers OD, Riis BJ, Pedersen S (1993) Bone turnover in asthmatic
11 children treated with oral prednisolone or inhaled budesonide. *Pediatr*
12 *Pulmonol* 16:341–346. <https://doi.org/10.1002/ppul.1950160604>
- 13 9. Paglia F, Dionisi S, De Geronimo S, Rosso R, Romagnoli E, Rajejntroph N,
14 Ragno A, Celi M, Pepe J, D'Erasmus E, Minisola S (2001) Biomarkers of
15 bone turnover after a short period of steroid therapy in elderly men. *Clin*
16 *Chem* 47:1314–1316.
- 17 10. Ahmed SF, Tucker P, Mushtaq T, Wallace AM, Williams DM, Hughes IA
18 (2002) Short-term effects on linear growth and bone turnover in children

1 randomized to receive prednisolone or dexamethasone. Clin Endocrinol
2 (Oxf) 57:185–191. <https://doi.org/10.1046/j.1365-2265.2002.01580.x>

3 11. Bath LF, Crofton PM, Evans AE, Ranke MB, Elmlinger MW, Kelnar CJ,
4 Wallace WH (2004) Bone turnover and growth during and after
5 chemotherapy in children with solid tumors. Pediatr Res 55:224–230.
6 <https://doi.org/10.1203/01.PDR.0000100903.83472.09>

7 12. Biyikli NK, Emre S, Sirin A, Bilge I (2004) Biochemical bone markers in
8 nephrotic children. Pediatr Nephrol 19:869–873.
9 <https://doi.org/10.1007/s00467-004-1537-6>

10 13. Dovio A, Perazzolo L, Osella G, Ventura M, Termine A, Milano E, Bertolotto
11 A, Angeli A (2004) Immediate fall of bone formation and transient increase
12 of bone resorption in the course of high-dose, short-term glucocorticoid
13 therapy in young patients with multiple sclerosis. J Clin Endocrinol Metab
14 89:4923–4928. <https://doi.org/10.1210/jc.2004-0164>

15 14. Brabnikova Maresova K, Pavelka K, Stepan JJ (2013) Acute effects of
16 glucocorticoids on serum markers of osteoclasts, osteoblasts, and
17 osteocytes. Calcif Tissue Int 92:354–361. [https://doi.org/10.1007/s00223-](https://doi.org/10.1007/s00223-012-9684-4)
18 [012-9684-4](https://doi.org/10.1007/s00223-012-9684-4)

- 1 15. Aceto G, D'Addato O, Messina G, Carbone V, Cavallo L, Brunetti G,
2 Faienza MF (2014) Bone health in children and adolescents with steroid-
3 sensitive nephrotic syndrome assessed by DXA and QUS. *Pediatr Nephrol*
4 29:2147–2155. <https://doi.org/10.1007/s00467-014-2834-3>
- 5 16. Vihinen MK, Kolho KL, Ashorn M, Verkasalo M, Raivio T (2008) Bone
6 turnover and metabolism in paediatric patients with inflammatory bowel
7 disease treated with systemic glucocorticoids. *Eur J Endocrinol* 159:693–
8 698. <https://doi.org/10.1530/EJE-08-0429>
- 9 17. Ekenstam E, Stålenheim G, Hällgren R (1988) The acute effect of high
10 dose corticosteroid treatment on serum osteocalcin. *Metabolism* 37:141–
11 144. [https://doi.org/10.1016/s0026-0495\(98\)90008-7](https://doi.org/10.1016/s0026-0495(98)90008-7)
- 12 18. Godschalk MF, Downs RW (1988) Effect of short-term glucocorticoids on
13 serum osteocalcin in healthy young men. *J Bone Miner Res* 3:113–115.
14 <https://doi.org/10.1002/jbmr.5650030117>
- 15 19. Crofton PM, Ahmed SF, Wade JC, Stephen R, Elmlinger MW, Ranke MB,
16 Kelnar CJ, Wallace WH (1998) Effects of intensive chemotherapy on bone
17 and collagen turnover and the growth hormone axis in children with acute
18 lymphoblastic leukemia. *J Clin Endocrinol Metab* 83:3121–3129.

1 <https://doi.org/10.1210/jcem.83.9.5133>

2 20. Iijima K, Sako M, Nozu K, Mori R, Tachida N, Kamei K, Miura K, Aya K,
3 Nakanishi K, Ohtomo Y, Takahashi S, Tanaka R, Kaito H, Nakamura H,
4 Ishikura K, Ito S, Ohashi Y (2014) Rituximab for childhood-onset,
5 complicated, frequently relapsing nephrotic syndrome or steroid-
6 dependent nephrotic syndrome: a multicentre, double-blind, randomised,
7 placebo-controlled trial. Lancet 384:1273–1281.
8 [https://doi.org/10.1016/s0140-6736\(14\)60541-9](https://doi.org/10.1016/s0140-6736(14)60541-9)

9 21. Kamei K, Ishikura K, Sako M, Aya K, Tanaka R, Nozu K, Kaito H, Nakanishi
10 K, Ohtomo Y, Miura K, Takahashi S, Morimoto T, Kubota W, Ito S,
11 Nakamura H, Iijima K; Rituximab for Childhood-Onset Refractory
12 Nephrotic Syndrome (RCRNS) Study Group (2017) Long-term outcome of
13 childhood-onset complicated nephrotic syndrome after a multicenter,
14 double-blind, randomized, placebo-controlled trial of rituximab. Pediatr
15 Nephrol 32:2071–2078. <https://doi.org/10.1007/s00467-017-3718-0>

16 22. Takahashi T, Okamoto T, Sato Y, Yamazaki T, Hayashi A, Aoyagi H, Ueno
17 M, Kobayashi N, Uetake K, Nakanishi M, Ariga T (2019) Periodically
18 repeated rituximab administrations in children with refractory nephrotic

syndrome: 2-year multicenter observational study. *Pediatr Nephrol* 34:87–96. <https://doi.org/10.1007/s00467-018-4063-7>

23. Takahashi T, Okamoto T, Sato Y, Hayashi A, Ueda Y, Ariga T (2020) Glucose metabolism disorders in children with refractory nephrotic syndrome. *Pediatr Nephrol* 35:649–657. <https://doi.org/10.1007/s00467-019-04360-1>

24. Takahashi T, Okamoto T, Yokota I, Sato Y, Hayashi A, Ueda Y, Aoyagi H, Ueno M, Kobayashi N, Uetake K, Nakanishi M, Ariga T (2022) The effect of rituximab on the quality of life of children with refractory nephrotic syndrome. *Pediatr Int* 64:e14725. <https://doi.org/10.1111/ped.14725>

25. Højsager FD, Rand MS, Pedersen SB, Nissen N, Jørgensen NR (2019) Fracture-induced changes in biomarkers CTX, PINP, OC, and BAP—a systematic review. *Osteoporos Int* 30:2381–2389. <https://doi.org/10.1007/s00198-019-05132-1>

26. Lips P, van Schoor NM (2011) The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab* 25:585–591. <https://doi.org/10.1016/j.beem.2011.05.002>

27. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis

1 Work Group (2012) KDIGO Clinical Practice Guideline for
2 Glomerulonephritis. *Kidney Int Suppl* 2:139–274.

3 28. (1981) The primary nephrotic syndrome in children. Identification of
4 patients with minimal change nephrotic syndrome from initial response to
5 prednisone. A report of the International Study of Kidney Disease in
6 Children. *J Pediatr* 98:561–564. [https://doi.org/10.1016/s0022-](https://doi.org/10.1016/s0022-3476(81)80760-3)
7 [3476\(81\)80760-3](https://doi.org/10.1016/s0022-3476(81)80760-3)

8 29. Isojima T, Kato N, Ito Y, Kanzaki S, Murata M (2016) Growth standard
9 charts for Japanese children with mean and standard deviation (SD)
10 values based on the year 2000 national survey. *Clin Pediatr Endocrinol*
11 *25*:71–76. <https://doi.org/10.1297/cpe.25.71>

12 30. Kato N, Takimoto H, Sudo N (2011) The cubic functions for spline
13 smoothed L, S and M values for BMI reference data of Japanese children.
14 *Clin Pediatr Endocrinol* 20:47–49. <https://doi.org/10.1297/cpe.20.47>

15 31. Uemura O, Nagai T, Ishikura K, Ito S, Hataya H, Gotoh Y, Fujita N, Akioka
16 Y, Kaneko T, Honda M (2014) Creatinine-based equation to estimate the
17 glomerular filtration rate in Japanese children and adolescents with
18 chronic kidney disease. *Clin Exp Nephrol* 18:626–633.

1 <https://doi.org/10.1007/s10157-013-0856-y>

2 32. Rauchenzauner M, Schmid A, Heinz-Erian P, Kapelari K, Falkensammer
3 G, Griesmacher A, Finkenstedt G, Högl W (2007) Sex- and age-specific
4 reference curves for serum markers of bone turnover in healthy children
5 from 2 months to 18 years. J Clin Endocrinol Metab 92:443–449.
6 <https://doi.org/10.1210/jc.2006-1706>

7 33. Crofton PM, Evans N, Taylor MR, Holland CV (2004) Procollagen type I
8 amino-terminal propeptide: pediatric reference data and relationship with
9 procollagen type I carboxyl-terminal propeptide. Clin Chem 50:2173–2176.
10 <https://doi.org/10.1373/clinchem.2004.039958>

11 34. Ishikura K, Matsumoto S, Sako M, Tsuruga K, Nakanishi K, Kamei K, Saito
12 H, Fujinaga S, Hamasaki Y, Chikamoto H, Ohtsuka Y, Komatsu Y, Ohta T,
13 Nagai T, Kaito H, Kondo S, Ikezumi Y, Tanaka S, Kaku Y, Iijima K;
14 Japanese Society for Pediatric Nephrology (2015) Clinical practice
15 guideline for pediatric idiopathic nephrotic syndrome 2013: medical
16 therapy. Clin Exp Nephrol 19:6–33. [https://doi.org/10.1007/s10157-014-](https://doi.org/10.1007/s10157-014-1030-x)
17 1030-x

18 35. Pocock NA, Eisman JA, Dunstan CR, Evans RA, Thomas DH, Huq NL

(1987) Recovery from steroid-induced osteoporosis. *Ann Intern Med* 107:319–323. <https://doi.org/10.7326/0003-4819-107-2-319>

36. Szappanos Á, Tőke J, Lippai D, Patócs A, Igaz P, Szücs N, Fütő L, Gláz E, Rácz K, Tóth M (2010) Bone turnover in patients with endogenous Cushing's syndrome before and after successful treatment. *Osteoporos Int* 21:637–645. <https://doi.org/10.1007/s00198-009-0978-y>

37. Gulati S, Godbole M, Singh U, Gulati K, Srivastava A (2003) Are children with idiopathic nephrotic syndrome at risk for metabolic bone disease? *Am J Kidney Dis* 41:1163–1169. [https://doi.org/10.1016/s0272-6386\(03\)00348-2](https://doi.org/10.1016/s0272-6386(03)00348-2)

38. Gulati S, Gulati K (2005) Bone disease in nephrotic syndrome--prevention is better than cure. *Pediatr Nephrol* 20:111–112. <https://doi.org/10.1007/s00467-004-1579-9>

39. Vestergaard P, Olsen ML, Paaske Johnsen S, Rejnmark L, Toft Sørensen H, Mosekilde L (2003) Corticosteroid use and risk of hip fracture: a population-based case-control study in Denmark. *J Intern Med* 254:486–493. <https://doi.org/10.1046/j.1365-2796.2003.01219.x>

40. Ton FN, Gunawardene SC, Lee H, Neer RM (2005) Effects of low-dose

prednisone on bone metabolism. J Bone Miner Res 20:464–470.

<https://doi.org/10.1359/JBMR.041125>

41. Bowden SA, Akusoba CI, Hayes JR, Mahan JD (2016) Biochemical markers of bone turnover in children with clinical bone fragility. J Pediatr Endocrinol Metab 29:715–722. <https://doi.org/10.1515/jpem-2014-0525>

42. Ward LM (2020) Glucocorticoid-induced osteoporosis: Why kids are different. Front Endocrinol (Lausanne) 11:576. <https://doi.org/10.3389/fendo.2020.00576>

43. Araya CE, Wasserfall CH, Brusko TM, Mu W, Segal MS, Johnson RJ, Garin EH (2006) A case of unfulfilled expectations. Cytokines in idiopathic minimal lesion nephrotic syndrome. Pediatr Nephrol 21:603–610. <https://doi.org/10.1007/s00467-006-0026-5>

44. Leonard MB (2007) Glucocorticoid-induced osteoporosis in children: impact of the underlying disease. Pediatrics 119 Suppl 2:S166–174. <https://doi.org/10.1542/peds.2006-2023J>

Figure Captions

Fig. 1 Scheme for BTM measurement and PSL tapering method. We administered rituximab every 4–6 months, and BTMs were measured at the same time as rituximab. When a patient took PSL at the time of rituximab administration, PSL was tapered off every two weeks after administration of rituximab. If we obtained a pair of BTMs at GC administration and after GC discontinuation, we assessed the changes in these BTMs. Daily PSL dose at GC administration was assessed. Abbreviations: BTMs, bone turnover markers; GC, glucocorticoid; PSL, prednisolone; RTX, rituximab

Fig. 2 Flow diagram of the patients. Abbreviations: BTMs, bone turnover markers; GH, growth hormone; RTX, rituximab

Fig. 3 Changes in BTMs after GC discontinuation stratified by daily PSL dose at GC administration. Changes in (a) ln BAP SD score, (b) log intact P1NP SD score, and (c) TRACP-5b are shown by daily PSL dose at GC administration for <0.25 mg/kg/day, 0.25–0.49 mg/kg/day, 0.50–0.99 mg/kg/day, and ≥1.0 mg/kg/day. *Pre*: at GC administration, *post*: after GC discontinuation. The number of pairs

analyzed are shown in the figure. For the group with a daily PSL dose <0.25 mg/kg/day at GC administration, changes in BTMs after GC discontinuation tend to be milder than those of the other groups. Abbreviations: BAP, bone alkaline phosphatase; BTMs, bone turnover markers; GC, glucocorticoid; PSL, prednisolone; P1NP, procollagen type 1 N-terminal propeptide; SD, standard deviation; TRACP-5b, tartrate-resistant acid phosphatase-5b

Fig. 4 Changes in BTMs after GC discontinuation stratified by daily PSL dose with a cutoff of 0.25 mg/kg/day at GC administration. Changes in (a) In BAP SD score, (b) log intact P1NP SD score, and (c) TRACP-5b are stratified according to daily PSL dose with a cutoff of 0.25 mg/kg/day. *Pre*: at GC administration, *post*: after GC discontinuation. Thirty-seven, 33, and 38 pairs of BTMs are analyzed for In BAP SD score, log intact P1NP SD score, and TRACP-5b, respectively. There are no significant changes in BTMs after discontinuation of PSL at a dose <0.25 mg/kg/day, whereas there are significant increases in BTMs after discontinuation of PSL at a dose ≥ 0.25 mg/kg/day. Abbreviations: BAP, bone alkaline phosphatase; BTMs, bone turnover markers; GC, glucocorticoid; PSL, prednisolone; P1NP, procollagen type 1 N-terminal propeptide; SD, standard

1 deviation; TRACP-5b, tartrate-resistant acid phosphatase-5b

Table 1 Baseline clinical characteristics of the patients (n = 29)

Sex	Female	11 (38%)
	Male	18 (62%)
Histopathology	MGA	19 (66%)
	FSGS	1 (3%)
	Biopsy not done	9 (31%)
History of SRNS	7 (24%)	
Number of relapses before baseline	1–10	12 (41%)
	11–20	8 (28%)
	21–30	3 (10%)
	NA	6 (21%)
Age at onset of NS, years old	3.8 (2.6–6.6)	
Age at baseline, years old	12.4 (9.0–14.5)	
Duration of NS before baseline, years	5.9 (3.3–9.7)	
Immunosuppressants until baseline	Cyclosporine	13 (45%)
	Cyclosporine and mizoribine	9 (31%)
	Mizoribine	7 (24%)
Height at baseline ^a , cm	135.8 ± 17.0	
Height SD score at baseline ^a	-1.26 ± 1.26	
Body weight at baseline ^a , kg	36.9 ± 10.5	
Body weight SD score at baseline ^a	-0.25 ± 1.24	
BMI at baseline ^a , kg/m ²	19.3 (16.5–20.8)	
BMI SD score at baseline ^a	0.43 ± 1.19	
Vitamin D ₃ administration at baseline	20 (69%)	

Categorical variables are shown as numbers (percentages). Continuous variables are shown as mean ± SD or median (interquartile range). The first time point of measurement of bone turnover markers at glucocorticoid administration to be analyzed in each patient is set as "baseline."

^a n = 28 due to insufficient data on height in a patient.

Abbreviations: BMI, body mass index; FSGS, focal segmental glomerulosclerosis; MGA, minor glomerular abnormalities; NA, not available; NS, nephrotic syndrome; SD, standard deviation; SRNS, steroid-resistant nephrotic syndrome

Table 2 Characteristics of the patients in the 38 pairs of BTM measurements at GC administration and after GC discontinuation

	At GC administration		After GC discontinuation	<i>P</i> value
Period after last PSL dose increase due to relapse ^a , days	40 (17–118)		—	—
Period after last PSL dose change ^b , days	15 (9–29)		—	—
Daily PSL dose ^c , mg/day	17.5 (4.7–31.3)		—	—
Daily PSL dose ^c , mg/kg/day	0.44 (0.11–0.98)		—	—
Administration method of PSL	Daily	16 (42%)	—	—
	On alternate days	22 (58%)		
Period after PSL discontinuation, months	—		3.5 ± 1.0	—
Vitamin D ₃ administration	25 (66%)		28 (74%)	—
Age, years old	12.8 (9.7–14.6)		13.3 (10.2–15.1)	< 0.01
Height ^d , cm	138.4 ± 16.4		141.2 ± 15.8	< 0.01
Height SD score ^d	-1.29 ± 1.15		-1.19 ± 1.09	< 0.01
Body weight ^d , kg	39.8 ± 11.8		39.5 ± 11.9	0.55
Body weight SD score ^d	-0.18 ± 1.22		-0.48 ± 1.18	< 0.01
BMI ^d , kg/m ²	19.9 (17.1–23.0)		18.7 (16.4–22.1)	< 0.01
BMI SD score ^d	0.50 ± 1.19		0.12 ± 1.15	< 0.01
Albumin, g/dL	4.0 ± 0.5		4.5 ± 0.2	< 0.01
Creatinine, mg/dL	0.42 ± 0.10		0.46 ± 0.12	< 0.01
Estimated GFR ^d , mL/min/1.73m ²	125.3 ± 19.5		121.1 ± 19.7	0.17
Adjusted calcium, mg/dL	9.6 (9.4–9.8)		9.7 (9.4–10.0)	0.23
Phosphate, mg/dL	4.0 ± 0.6		4.4 ± 0.5	< 0.01
Intact PTH, pg/mL	41 ± 20		32 ± 17	< 0.01

Categorical variables are shown as numbers (percentages). Continuous variables are shown as mean ± SD or median (interquartile range).

^a n = 34 due to insufficient data on PSL administration in four pairs.

^b n = 35 due to insufficient data on PSL administration in three pairs. PSL dose change means an increase of PSL dose due to relapse or a reduction of PSL dose as a tapering process.

^c When PSL was administered on alternate days, the value divided by two was taken as the daily PSL dose (e.g., when PSL 30 mg was administered on alternate days, then the daily PSL dose was 15 mg/day).

^d n = 37 due to insufficient data on height in a pair.

Abbreviations: BMI, body mass index; BTMs, bone turnover markers; GC, glucocorticoid; GFR, glomerular filtration rate; PSL,

Table 3 Changes in BTMs after GC discontinuation

	At GC administration	After GC discontinuation	<i>P</i> value
BAP ^a , µg/L	56.1 (31.0–74.5)	96.4 (57.4–109.6)	< 0.01
ln BAP SD score ^a	0.06 (-0.71–0.93)	1.43 (0.89–1.79)	< 0.01
Intact P1NP ^b , µg/L	170.6 (97.1–415.1)	476.3 (314.7–703.2)	< 0.01
log intact P1NP SD score ^b	-2.78 (-4.36–2.74)	0.11 (-0.58–1.22)	< 0.01
TRACP-5b ^c , U/L	16.1 ± 5.6	20.8 ± 8.2	< 0.01

Continuous variables are shown as mean ± SD or median (interquartile range).

^a Analyzed in 37 pairs due to a lack of data in a pair.

^b Analyzed in 33 pairs due to a lack of data in five pairs.

^c Analyzed in 38 pairs.

Abbreviations: BAP, bone alkaline phosphatase; BTMs, bone turnover markers; GC, glucocorticoid; P1NP, procollagen type 1 N-terminal propeptide; SD standard deviation; TRACP-5b, tartrate-resistant acid phosphatase-5b

Table 4 Correlations between changes in factors that can affect bone turnover and changes in BTMs after GC discontinuation

	Change in ln BAP SD score ^a		Change in log intact P1NP SD score ^b		Change in TRACP-5b (U/L) ^c	
Daily PSL dose (mg/kg/day) at GC administration	$r = 0.38$	$p < 0.05$	$r = 0.56$	$p < 0.01$	$r = 0.33$	$p < 0.05$
Change in height SD score	$r = 0.10$	$p = 0.56$	$r = 0.20$	$p = 0.27$	$r = 0.24$	$p = 0.14$
Change in albumin (g/dL)	$r = 0.16$	$p = 0.36$	$r = 0.29$	$p = 0.10$	$r = 0.14$	$p = 0.39$
Change in phosphate (mg/dL)	$r = -0.05$	$p = 0.79$	$r = 0.21$	$p = 0.24$	$r = 0.19$	$p = 0.25$
Change in intact PTH (pg/mL)	$r = -0.04$	$p = 0.83$	$r = -0.26$	$p = 0.15$	$r = -0.24$	$p = 0.14$

^a Analyzed in 37 pairs due to a lack of data in a pair.

^b Analyzed in 33 pairs due to a lack of data in five pairs.

^c Analyzed in 38 pairs.

Abbreviations: BAP, bone alkaline phosphatase; BTMs, bone turnover markers; GC, glucocorticoid; PSL, prednisolone; PTH, parathyroid hormone; P1NP, procollagen type 1 N-terminal propeptide; SD standard deviation; TRACP-5b, tartrate-resistant acid phosphatase-5b

Figure 1

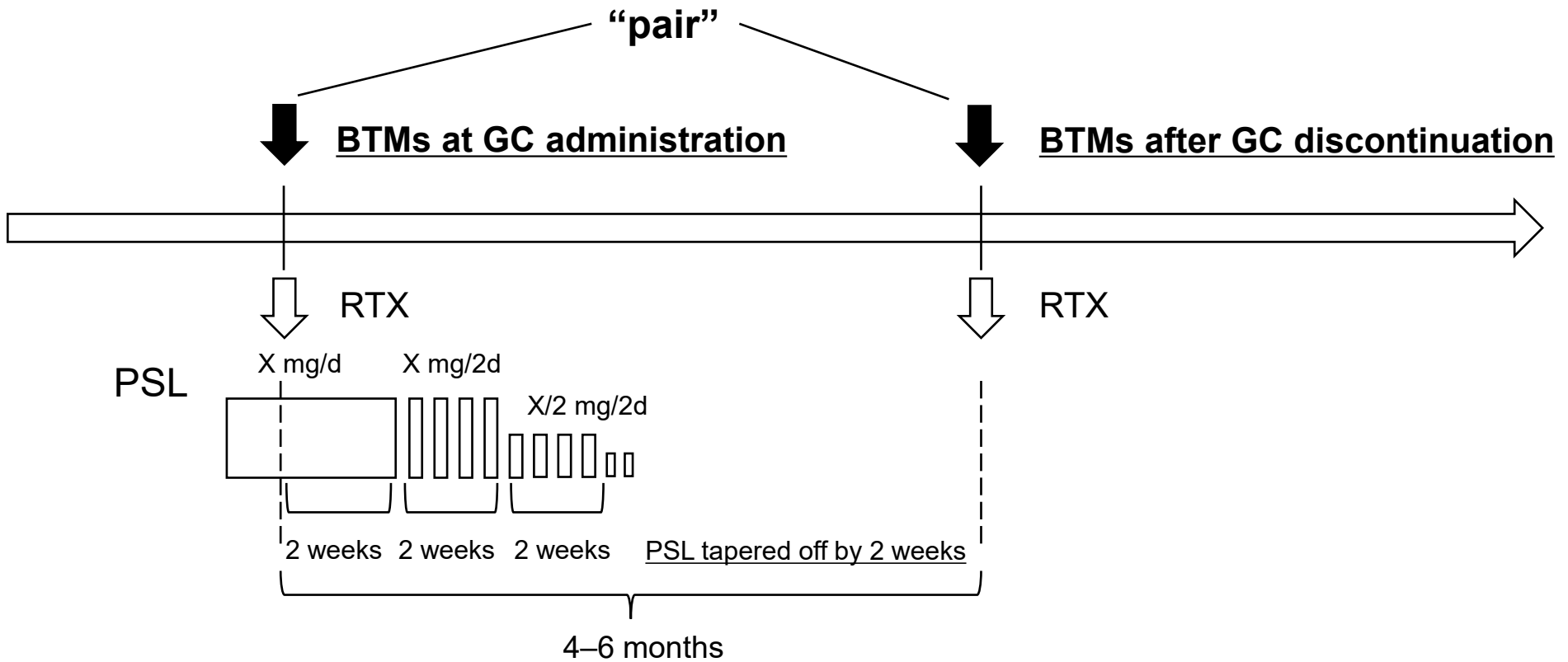


Figure 2

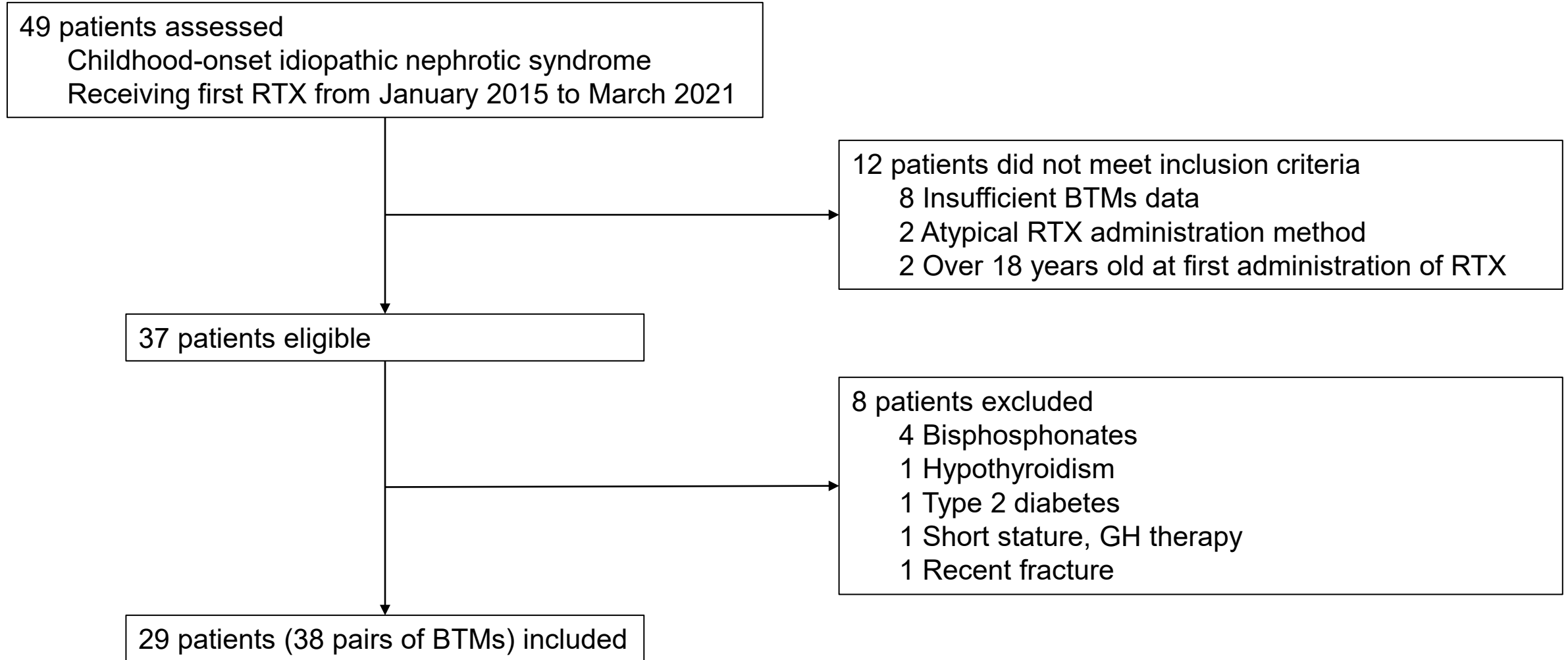


Figure 3

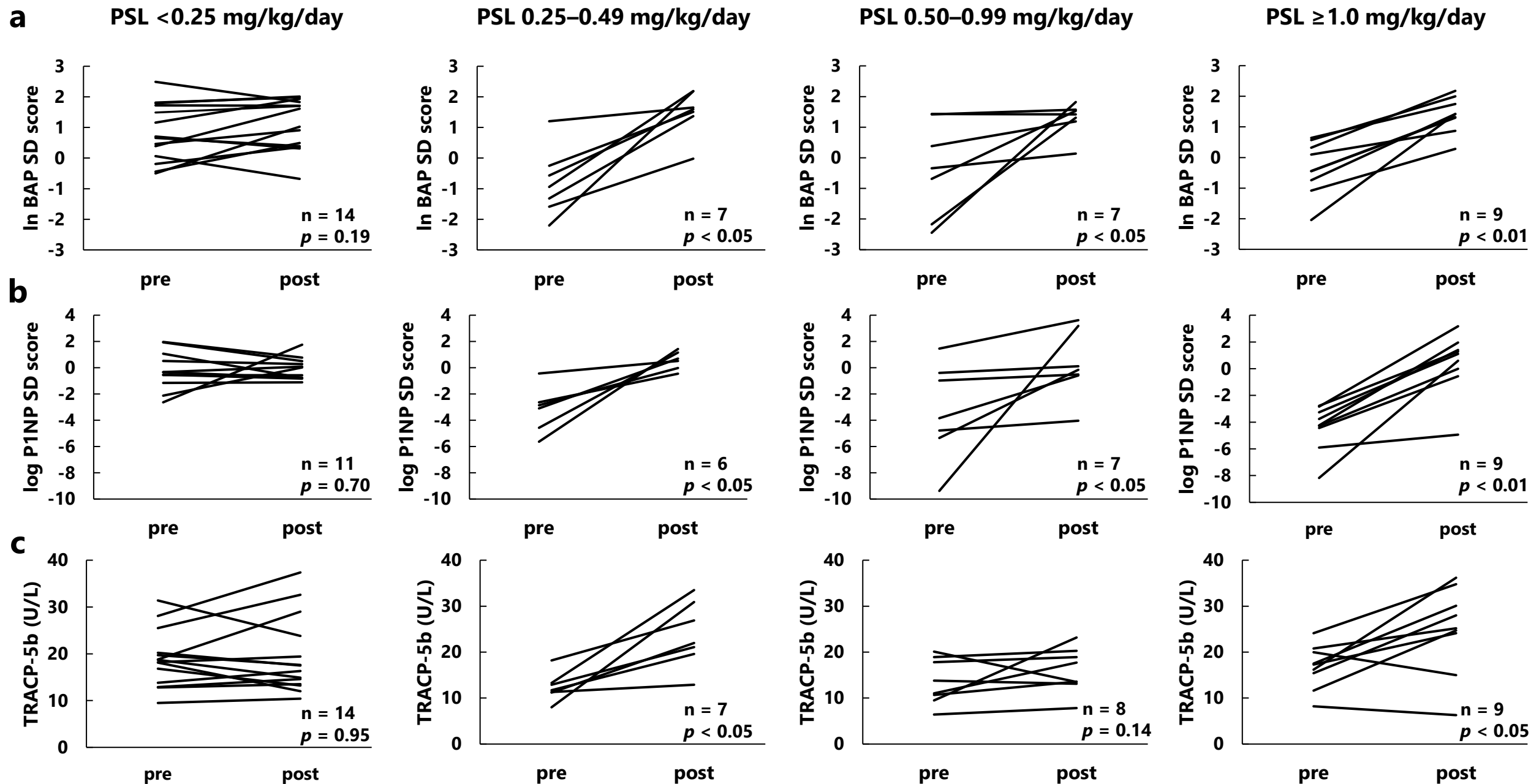


Figure 4

