Supplemental material

Effects of the Potassium-Binding Polymer Patiromer on Markers of Mineral Metabolism

David A. Bushinsky, MD, David M. Spiegel, MD, Jinwei Yuan, MD, Suzette Warren, Jeanene Fogli, PhD, Pablo E. Pergola, MD, PhD

Table of Contents

Appendix 1. Serum chemistry methods used in the TOURMALINE study.

Chemistry Panels

Blood samples for chemistry panels (including serum calcium, magnesium, and inorganic phosphate) were collected in 7.0 mL serum separator tubes, filled completely, inverted 5 times to mix, and kept in the upright position for 45 minutes to allow clotting. Within 60 minutes of blood collection, tubes were then centrifuged for 15 minutes at 1800 g to separate serum and cells. Tubes were allowed to sit upright for 1 hour prior to shipment (at ambient temperature, 15–25°C) to the central laboratory (Medpace Reference Laboratories, Cincinnati, Ohio) for analysis. Serum analytes were detected using a Beckman Coulter AU analyzer.

Serum calcium level was determined using the Beckman Coulter Calcium oCPC (o-cresolphthalein complexone) reagent kit, via measurement of the complex formed between calcium and oCPC using absorption spectroscopy. The manufacturer-reported analytical measuring range of the assay is 0.0–18.0 mg/dL, with coefficients of variation <2%.

Serum magnesium level was determined using the Beckman Coulter Magnesium Reagent kit, via measurement of the complex formed between magnesium and xylidyl blue at pH 11.4 using absorption spectroscopy. The manufacturer-reported analytical measuring range of the assay is 0.5–8.0 mg/dL, with coefficients of variation <2%.

Serum phosphate level was determined using the Beckman Coulter Inorganic Phosphorous Reagent kit, via measurement of the heteropolyacid complex formed between phosphate and molybdate using absorption spectroscopy. The manufacturer-reported analytical measuring range of the assay is 1.0–20.0 mg/dL, with coefficients of variation <2%.

Serum Potassium

Blood samples for measurement of serum potassium by the central laboratory were collected in 4.0 mL serum separator tubes, filled completely, inverted 5 times to mix, and kept in the upright position for 15–30 minutes to allow clotting. Within 60 minutes of blood collection, tubes were then centrifuged for 15 minutes at 1800 g to separate serum and cells. Serum was aliquoted into a false-bottom vial for shipment at ambient temperature (15–25°C). Potassium was assayed by the central laboratory using an ion-selective electrode on an AU Beckman chemistry analyzer with an internal reference solution. The manufacturer-reported analytical measuring range of the assay is 1.0-10.0 mmol/L, with coefficients of variation <5%.

In order to identify samples with artifactual potassium readings due to hemolysis, a lipemia-icterus-hemolysis assay was used to determine free hemoglobin in serum. Samples identified as positive for hemolysis were not analyzed for serum potassium, and the value was coded as missing in the database.

For local serum potassium samples, blood was collected in 1.0 mL LiHeparin tubes, filled completely and inverted 8–10 times before pipetting whole blood (not centrifuged) into an i-STAT Point-of-Care device cartridge (Abbott Laboratories, Abbott Park, IL) for potassium measurement.

Intact parathyroid Hormone

Blood samples for analysis of intact parathyroid hormone (iPTH) were collected in 2.0 mL K2 EDTA tubes, filled completely, inverted 8–10 times to mix, and centrifuged at 1200 g for 15 minutes. Plasma was aliquoted into a cryovial and frozen at –15°C to –25°C before shipping in dry ice. iPTH levels were assayed on a Roche E-170 Modular analyzer using the Roche serum iPTH assay (F. Hoffmann-La Roche Ltd, Basel, Switzerland), a 2-step electrochemiluminescence immunoassay. The manufacturer-reported analytical measuring range of the assay is 1.20-5000 pg/mL, with a lowest calculated limit of detection of 1.20 pg/mL, and coefficients of variation <5%.

Vitamin D

Vitamin D levels were assayed on a Roche E-170 Modular analyzer using the Roche total vitamin D assay (F. Hoffmann-La Roche Ltd, Basel, Switzerland), an electrochemiluminescence immunoassay. The manufacturer-reported analytical measuring range of the assay is 3 – 70 ng/mL, with a lowest calculated limit of detection of 5 ng/mL, an intra-assay coefficient of variation <5%, and an inter-assay coefficient of variation <10%.

Fibroblast Growth Factor 23

Blood samples for measurement of intact fibroblast growth factor 23 (FGF-23) were collected in 2.5 mL serum separator tubes, filled completely, inverted 5 times to mix, and kept in the upright position for 45 minutes to allow clotting. Tubes were then centrifuged for 15 minutes at 1800 g to separate serum and cells. Within 60 minutes of blood collection, serum was aliquoted into a transfer vial and frozen at –15°C or colder before shipping in dry ice. FGF23 levels were

measured via an enzyme-linked immunosorbent assay (Kainos Laboratories, Tokyo, Japan). The manufacturer-reported analytical measuring range of the assay is 3–800 pg/mL, with Intra-assay coefficient of variation <5%, and inter-assay coefficient of variation <10%.

Urine Calcium and Phosphate

Measurement of calcium and phosphate from urine samples was performed using the same assays as for serum samples (described above).

Supplemental Table 1. Overall unadjusted and albumin-corrected serum calcium.

	Baseline		Week 4		Change from baseline to Week 4		
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	P-value[†]
Unadjusted serum calcium (mg/dL)	9.3 (0.6)	112	9.3 (0.6)	100	0.0 (0.5)	100	0.71
Albumin-corrected serum calcium* (mg/dL)	9.4 (0.6)	112	9.5 (0.6)	100	0.0 (0.5)	100	0.78

*Serum albumin-corrected calcium performed according to the following: If serum albumin ≥4.0 g/dL, corrected calcium=serum calcium; If serum albumin <4.0 g/dL, corrected calcium=serum calcium + (0.8 x [4 - serum albumin]). For calculations calcium units were mg/dL and albumin units were g/dL.

[†]Paired t-test for change from baseline.

Supplemental Table 2. Albumin-corrected serum calcium and serum phosphate week 4 change from baseline for the overall study group, and by randomized treatment group.

	Baseline		Week 4		Change from baseline to Week 4		
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N	P-value[†]
Albumin-corrected serum calcium, mg/dL*							
Overall	9.4 (0.6)	112	9.5 (0.6)	100	0.0 (0.5)	100	0.78
By randomized treatment group							
With food	9.4 (0.6)	55	9.5 (0.6)	49	0.0 (0.5)	49	0.95
Without food	9.4 (0.6)	57	9.4 (0.6)	51	0.0 (0.5)	51	0.76
Serum phosphate, mg/dL							
Overall	4.1 (0.7)	112	4.0 (0.7)	100	-0.1 (0.7)	100	0.47
By randomized treatment group							
With food	4.0 (0.8)	55	3.9 (0.7)	49	0.0 (0.7)	49	0.75
Without food	4.2 (0.7)	57	4.1 (0.7)	51	–0.1 (0.7)	51	0.49

*Serum albumin-corrected calcium performed according to the following: If serum albumin ≥4.0 g/dL, corrected calcium=serum calcium; If serum albumin <4.0 g/dL, corrected calcium=serum calcium + (0.8 x [4 - serum albumin]). For calculations calcium units were mg/dL and albumin units were g/dL.

[†]Paired t-test for change from baseline.

Supplemental Table 3. 24-hour creatinine-normalized urine calcium and phosphate week 4 change from baseline for the overall study group, and by randomized treatment group.

	Baseline		Week 4		Change from baseline to Week 4		
	Mean (SD)	N	Mean (SD)	N	Median (Q1, Q3)	N	P-value*
24-hour creatinine-normalized urine calcium, mg/24 hours							
Overall	50.8 (55.1)	73	58.5 (59.4)	78	2.5 (–11.5, 23.7)	69	0.10
By randomized treatment group							
With food	53.8 (55.3)	37	57.9 (50.3)	41	4.8 (–12.7, 25.8)	37	0.38
Without food	47.8 (55.5)	36	59.1 (68.9)	37	0.8 (–10.7, 21.1)	32	0.12
24-hour creatinine-normalized urine phosphate, mg/24 hours							
Overall	628.2 (276.0)	96	573.6 (286.2)	95	–43.0 (–162.6, 35.7)	95	0.004
By randomized treatment group							
With food	596.2 (281.3)	49	544.4 (284.8)	49	–75.0 (–159.6, 35.7)	49	0.05
Without food	661.6 (269.2)	47	604.7 (287.6)	46	–40.2 (–162.6, 33.4)	46	0.03

*Paired t-test for change from baseline.

Supplemental Table 4. 24-hour urine calcium/creatinine and phosphate/creatinine ratio week 4 change from baseline for the overall study group, and by randomized treatment group.

	Baseline		Week 4		Change from baseline to Week 4		
	Mean (SD)	N	Mean (SD)	N	Median (Q1, Q3)	N	P-value*
24-hour urine calcium/creatinine ratio, mg/g							
Overall	41.4 (43.8)	85	51.7 (58.3)	80	2.1 (–10.7, 26.3)	69	0.04
By randomized treatment group							
With food	47.8 (44.1)	42	57.0 (64.6)	42	4.6 (–12.9, 29.1)	37	0.11
Without food	35.1 (43.2)	43	46.0 (50.7)	38	0.9 (–10.0, 18.7)	32	0.16
24-hour urine phosphate/creatinine ratio, mg/g							
Overall	507.2 (150.2)	108	462.7 (163.1)	97	–43.6 (–133.0, 39.7)	95	0.002
By randomized treatment group							
With food	506.3 (165.8)	53	461.1 (166.4)	50	–63.1 (–133.0, 40.6)	49	0.04
Without food	508.0 (134.9)	55	464.5 (161.3)	47	–39.2 (–111.5, 32.5)	46	0.03

*Paired t-test for change from baseline.

Supplemental Table 5. Serum markers of mineral metabolism in patients with baseline serum phosphate >4.8 mg/dL (hyperphosphatemia) and baseline serum phosphate ≤4.8 mg/dL.

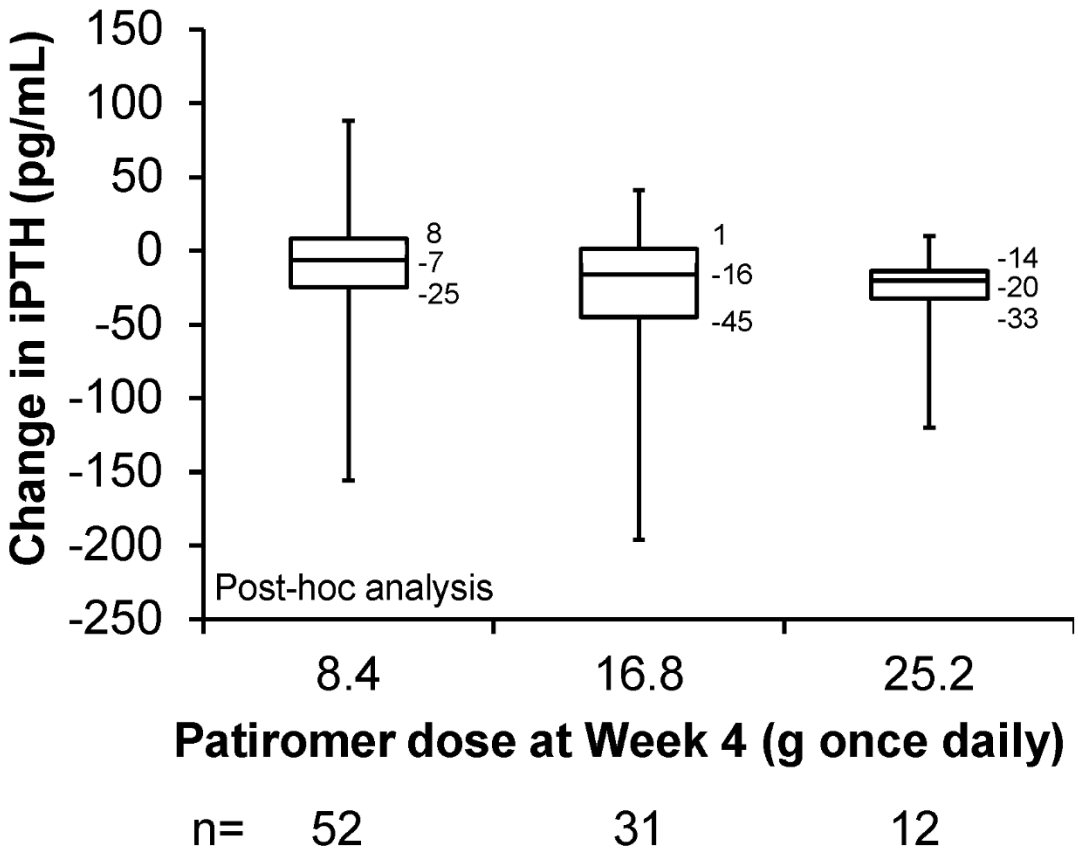
	Baseline		Week 4		Change from baseline to Week 4	
	Mean (SD)	N	Mean (SD)	N	Mean (SD)	N
Baseline serum phosphate >4.8 mg/dL						
Fibroblast growth factor 23, pg/mL	483 (587)	16	470 (696)	13	–27 (89)	13
Intact parathyroid hormone, pg/mL	137 (82)	14	93 (48)	13	–21 (50)	11
Baseline serum phosphate ≤4.8 mg/dL						
Fibroblast growth factor 23, pg/mL	98 (62)	94	104 (72)	86	8 (42)	86
Intact parathyroid hormone, pg/mL	79 (52)	95	61 (43)	86	–15 (30)	86

Supplemental Table 6. Shifts in serum electrolytes from baseline to end of treatment.

Parameter	End of Treatment, No. of patients (N=110)*		
Baseline	Low*	Normal	High†
Calcium‡			
Low (<8.5 mg/dL)	5	3	0
Normal	3	84	5
High (>10.2 mg/dL)	0	7	3
Magnesium			
Low (<1.8 mg/dL)	11	2	0
Normal	11	68	0
High (>2.4 mg/dL)	0	12	6
Phosphate			
Low (<2.5 mg/dL)	0	0	0
Normal	0	86	9
High (>4.8 mg/dL)	0	9	6

* Three patients in the safety population who had no post-baseline laboratory measurement (including one patient with hyperphosphatemia at baseline) were not included in this table.
†Same thresholds as at baseline, which are shown in the leftmost column.
‡Serum albumin-corrected calcium performed according to the following: If serum albumin ≥4.0 g/dL, corrected calcium=serum calcium; If serum albumin <4.0 g/dL, corrected calcium=serum calcium + (0.8 x [4 - serum albumin]). For calculations calcium units were mg/dL and albumin units were g/dL.

Supplemental Figure 1. Median intact parathyroid hormone changes from baseline to week 4 by patiromer dose.



Boxes represent median (center line), Q1 (lower bound), and Q3 (upper bound). Whiskers represent minimum and maximum values. iPTH = intact parathyroid hormone.