SUPPLEMENTARY APPENDIX

Effects of dietary phosphate restriction and phosphate binders on FGF23 levels in CKD

RUNNING TITLE: **Diet, binders and FGF23 in CKD**

This trial was registered at clinicaltrials.gov on February 12, 2009 as NCT00843349.

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Supplementary Methods

Measurements and assays

Standard assays were used for serum and urinary phosphate, calcium and creatinine. C-terminal FGF23 levels were measured by ELISA (Immutopics, San Clemente, CA; CV<5%), and intact PTH was measured using a chemiluminescent immunoassay (Roche Diagnostics, Indianapolis, IN; CV <3%). Fractional excretion of mineral (phosphate, FePi; calcium, FeCa) was calculated from spot urine collections as: (urine mineral x serum creatinine x 100)/(serum mineral x urine creatinine). 1,25-dihydroxyvitamin D levels were measured at baseline and at weeks 2 and 12 using liquid chromatography tandem mass spectrometry (Quest Diagnostics, San Juan Capistrano, CA).

Echocardiography

As a secondary exploratory outcome, we measured cardiac structure and function at baseline and at week 12 using transthoracic echocardiography (ie33, Phillips Healthcare, Andover, MA). Two-dimensional, pulsed-Doppler and color tissue Doppler imaging were performed from standard parasternal and apical transducer positions with 2-dimensional frame rates of 60-100 frames/second and tissue Doppler frame rates >100 frames/second. All data were stored digitally, and off-line data analysis was performed by a single reviewer (RBW), who was blinded to randomized group assignments. Cardiac structural measurements were made in accordance with current guidelines (1). Left ventricular ejection fraction (LVEF) was determined using the Teichholz formula from linear dimensions. In addition to body surface area–indexed left ventricular mass (LVM), we calculated LVM indexed to height$^{2.71}$ according to the modified American Society of Echocardiography equation (2). Relative wall thickness (RWT) was

calculated as posterior wall plus septal wall thicknesses divided by end-diastolic cavity dimension. Longitudinal tissue velocities were measured from pulsed-waved tissue Doppler image recordings and are reported as the average of three consecutive cardiac cycles.

Supplementary Table 1. Effects of dietary intervention and LC on fractional excretion of phosphate and calcium.

	Baseline	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12	P value
FECa, %								
Ad lib diet-LC placebo	0.06 ± 0.03	0.06 ± 0.02	0.07 ± 0.03	0.07 ± 0.04	0.07 ± 0.03	0.07± 0.03	0.07± 0.03	0.70
900 mg-Phosphate Diet-LC Placebo	0.08 ± 0.03	0.07 ± 0.03	0.09 ± 0.04	0.09 ± 0.04	0.09 ± 0.04	0.08 ± 0.03	0.08 ± 0.03	0.50
Ad lib Diet-LC	0.08 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.07 ± 0.03	0.06 ± 0.02	0.08 ± 0.03	0.06 ± 0.02	0.06
900 mg-Phosphate Diet-LC	0.09 ± 0.06	0.07 ± 0.04	0.08 ± 0.04	0.09 ± 0.05	0.08 ± 0.04	0.08 ± 0.06	0.09 ± 0.06	0.20
FEPi, %								
Ad lib diet-LC placebo	0.19 ± 0.07	0.17 ± 0.05	0.18 ± 0.07	0.19 ± 0.08	0.19 ± 0.07	0.19 ± 0.07	0.19 ± 0.07	0.84
900 mg-Phosphate Diet-LC Placebo	0.20 ± 0.05	0.20 ± 0.06	0.20 ± 0.06	0.21 ± 0.07	0.21 ± 0.06	0.20 ± 0.06	0.20 ± 0.06	0.80
Ad lib Diet-LC	0.19 ± 0.06	0.20 ± 0.07	0.19 ± 0.07	0.19 ± 0.07	0.17 ± 0.05	0.20 ± 0.06	0.18 ± 0.06	0.16
900 mg-Phosphate Diet-LC	0.24 ± 0.12	0.23 ± 0.08	0.22 ± 0.08	0.23 ± 0.10	0.23 ± 0.10	0.24 ± 0.13	0.24 ± 0.13	0.72

Mean values ± standard deviations are shown. P values are for within-group changes.

Supplementary Table 2. Effects of dietary intervention and LC on echocardiographic measurements.

	Baseline	End of Study	P
LVEF (%)			
Ad lib diet-LC placebo	60.6 ± 9.6	60.8 ± 11.5	0.93
900 mg-Phosphate Diet-LC Placebo	62.1 ± 9.2	59.8 ± 7.8	0.39
Ad lib Diet-LC	61.7 ± 5.4	61.5 ± 5.7	0.87
900 mg-Phosphate Diet-LC	55.4 ± 16.4	56.3 ± 12.5	0.52
LVMI indexed to BSA (g/m^2)			
Ad lib diet-LC placebo	71.6 ± 19.5	72.1 ± 27.8	0.91
900 mg-Phosphate Diet-LC Placebo	76.2 ± 14.4	77.9 ± 16.4	0.56
Ad lib Diet-LC	74.9 ± 10.0	70.1 ± 9.5	0.06
900 mg-Phosphate Diet-LC	74.7 ± 14.2	67.6 ± 16.5	0.27
LVMI indexed to height$^{2.71}$ ($g/m^{2.71}$)			
Ad lib diet-LC placebo	34.8 ± 10.6	35.0 ± 13.7	0.92
900 mg-Phosphate Diet-LC Placebo	38.0 ± 8.9	38.6 ± 8.8	0.46
Ad lib Diet-LC	36.0 ± 5.6	33.9 ± 7.1	0.11
900 mg-Phosphate Diet-LC	36.3 ± 9.2	33.0 ± 9.7	0.27
RWT			
Ad lib diet-LC placebo	0.43 ± 0.11	0.41 ± 0.09	0.32
900 mg-Phosphate Diet-LC Placebo	0.47 ± 0.15	0.47 ± 0.13	0.57
Ad lib Diet-LC	0.47 ± 0.10	0.40 ± 0.09	0.004
900 mg-Phosphate Diet-LC	0.41 ± 0.08	0.39 ± 0.08	0.75
E' lateral (cm/s)			
Ad lib diet-LC placebo	– 10.9 ± 4.2	– 10.7 ± 3.8	0.60
900 mg-Phosphate Diet-LC Placebo	– 8.8 ± 1.8	– 9.3 ± 1.7	0.22
Ad lib Diet-LC	– 10.2 ± 1.8	– 10.8 ± 3.7	0.58
900 mg-Phosphate Diet-LC	– 9.2 ± 2.5	– 9.1 ± 2.3	0.87
E' septum (cm/s)			
Ad lib diet-LC placebo	– 8.0 ± 2.6	– 7.9 ± 2.0	0.91
900 mg-Phosphate Diet-LC Placebo	– 6.6 ± 1.7	– 7.4 ± 1.0	0.13
Ad lib Diet-LC	– 6.9 ± 1.6	– 8.2 ± 3.2	0.08
900 mg-Phosphate Diet-LC	– 7.3 ± 2.7	– 7.3 ± 2.4	0.89

BSA, body surface area; LVEF, left ventricular ejection fraction; LVMI, left ventricular mass index; RWT, relative wall thickness; E' lateral, early diastolic peak tissue velocity at the lateral mitral annulus; E' septum, early diastolic peak tissue velocity at the septal mitral annulus

All values are means ± standard deviation. P values are for within-group changes.

References

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