

Supplement Table 1. Composition of the four diets

	1-AIN76A (Control)		2-Control+minerals		3-HFWD		4-HFWD+minerals	
	gm%	kcal%	gm%	kcal%	gm%	kcal%	gm%	kcal%
Protein	20.3	20.8	19.1	20.8	24.4	20.5	22.9	20.5
Carbohydrate	66	67.7	66	67.7	48.5	41.8	45.7	41.8
Fat	5	11.5	5	11.5	20	37.8	18.8	37.8
Total	91.3	100	86	100	92.9	100	87.5	100
kcal/gm	3.9		3.67		4.76		4.49	

INGREDIENTS	gm	kcal	gm	kcal	gm	kcal	gm	kcal
Casein (80 Mesh) ^a	200	800	200	800	240	960	240	960
DL-Methionine	3	12	3	12	0	0	0	0
L-Cystine	0	0	0	0	3.6	14	3.6	14
Corn starch	150	600	150	600	100	400	100	400
Maltodextrin 10	0	0	0	0	75	300	75	300
Sucrose	500	2000	500	2000	310.418	1242	310.418	1242
Cellulose (BW200)	50	0	50	0	20	0	20	0
Corn oil	50	450	50	450	200	1800	200	1800
Ethoxyquin	0.01	0	0.01	0	0.01	0	0.01	0
Mineral Mix for AIN76A ^b	35	0	35	0	0	0	0	0
Mineral Mix (without Calcium Phosphate) ^c	0	0	0	0	21	0	21	0
Monosodium phosphate	0	0	0	0	7.98	0	7.98	0
Monopotassium phosphate	0	0	0	0	7.91	0	7.91	0
Calcium carbonate (40% calcium)	0	0	0	0	0.88	0	0.88	0
Mineral supplement (12% calcium)	0	0	62	0	0	0	62	0
Vitamin Mix for AIN76A	10	40	10	40	0	0	0	0
Vitamin Mix (without Vit. D3 or Folic Acid)	0	0	0	0	12	48	12	48
Choline bitartrate	2	0	2	0	1.2	0	1.2	0
Folic acid	0	0	0	0	0.00023	0	0.00023	0
Vitamin D3 (100,000 IU/g)	0	0	0	0	0.0012	0	0.0012	0
TOTAL	1000.01	3902	1062.06	3902	1000.05	4764	1062.05	4764

^aAmount of essential minerals in Casein added to all diets (amount per 100 gm of Casein): calcium (25 mg), magnesium (2 mg), copper (0.01 mg), Iron (0.4 mg), manganese (0.014 mg), potassium (2.5 mg), selenium (0.03 mg), sodium (5.2 mg), zinc (4.2mg).

^bAmount of essential minerals in mineral mix for low fat diets with/without mineral supplement (used at 35 gm/kg diet): calcium (5.2 gm), magnesium (0.5 gm), chromium (2 mg), copper (6 mg), Iron (45 mg), manganese (59 mg), potassium (3.6 g), selenium (0.16 mg), sodium (1.0 gm), zinc (29 mg).

^cAmount of essential minerals in mineral mix for western-style diets with/without mineral supplement (used at 21 gm/kg): magnesium (0.6 gm), chromium (2.4 mg), copper (7.2 mg), Iron (54 mg), manganese (71 mg), potassium (4.3 g), selenium (0.19 mg), sodium (1.2 gm), zinc (35 mg).

Diets were formulated by Research Diets Incorporated (New Brunswick, NJ).

**Supplement Table 2. Minerals concentration in diets (mg/kg)
(as present in the diets from all sources)**

Mineral	Control	Control+Minerals	HFWD	HFWD+Minerals
Calcium	5250	11950	410	7360
Magnesium	504	1362	605	1457
Sodium	1010	1088	1212	1279
Potassium	3605	3402	4326	4081
Copper	6.02	5.74	7.2	6.9
Chromium	2	1.93	2.4	2.3
Iron	46	53	55	61
Manganese	59	58	71	69
Selenium	0.22	0.44	0.26	0.48
Strontium	<0.05	40	<0.05	40
Zinc	37	36	45	43

Diets were formulated and mineral calculations were provided by Research Diets Incorporated (New Brunswick, NJ).

Supplement Table 3. Average weight by diet group at 5, 12 and 18 months

Group	Weight (gm)		
	5 months	12 months	18 months
Control	25 ± 2	33 ± 2	35 ± 4
Control + minerals	24 ± 2	31 ± 5	32 ± 5
HFWD	28 ± 5	46 ± 6	47 ± 6
HFWD + minerals	31 ± 4	44 ± 8	49 ± 7

Values are means and standard deviations. Zero time (3 weeks of age) average weight was 14.5±1 gm.

Supplement Table 4. Micro-CT analysis of cortical and trabecular regions of femora from **female** mice in four diet groups at **five** months

	CONTROL	CONTROL + minerals	HFWD	HFWD + minerals
Cortical				
Bone mineral content (mg)	3.9 ± 0.2	4.2 ± 0.2 ^c	3.6 ± 0.3	4.0 ± 0.2 ^a
Bone mineral density (mg/cc)	347 ± 16	378 ± 25 ^c	314 ± 36	339 ± 16
Tissue mineral density (mg/cc)	1136 ± 24	1140 ± 12	1030 ± 77	1123 ± 15 ^a
Mean thickness (mm)	0.215 ± 0.007	0.225 ± 0.007 ^c	0.188 ± 0.030	0.225 ± 0.008 ^{a, b}
Polar moment of inertia (mm ⁴)	0.133 ± 0.008	0.147 ± 0.011	0.142 ± 0.024	0.147 ± 0.013
Endosteal perimeter (mm)	3.6 ± 0.2	3.8 ± 0.1	3.9 ± 0.2	3.7 ± 0.1 ^a
Periosteal perimeter (mm)	5.0 ± 0.2	5.2 ± 0.2	5.1 ± 0.3	5.1 ± 0.1
Marrow area (mm ²)	0.90 ± 0.07	0.94 ± 0.06	1.07 ± 0.12	0.94 ± 0.07 ^a
Cross sectional area (mm ²)	0.86 ± 0.02	0.92 ± 0.03 ^c	0.79 ± 0.13	0.92 ± 0.02 ^{a, b}
Total area (mm ²)	1.76 ± 0.07	1.85 ± 0.08	1.86 ± 0.16	1.86 ± 0.06
Trabecular				
Bone mineral content (mg)	0.51 ± 0.04	0.74 ± 0.06 ^c	0.59 ± 0.15	0.65 ± 0.05 ^b
Bone mineral density (mg/cc)	156 ± 12	234 ± 15 ^c	164 ± 54	201 ± 11 ^{a, b}
Tissue mineral density (mg/cc)	577 ± 55	596 ± 30	527 ± 20	581 ± 13 ^a
Bone volume fraction (mm ³ /mm ³)	0.05 ± 0.01	0.13 ± 0.02 ^c	0.05 ± 0.01	0.10 ± 0.02 ^{a, b}
Surface to volume ratio (mm ² /mm ³)	60.0 ± 10.0	49.6 ± 3.7 ^c	55.6 ± 3.5	49.6 ± 4.0 ^{a, b}
Trabecular thickness (mm)	0.034 ± 0.007	0.041 ± 0.003 ^c	0.036 ± 0.002	0.041 ± 0.003 ^{a, b}
Trabecular number (1/mm)	1.36 ± 0.25	3.30 ± 0.28 ^c	1.47 ± 0.29	2.50 ± 0.31 ^{a, b}
Trabecular spacing (mm)	0.67 ± 0.09	0.26 ± 0.03 ^c	0.66 ± 0.12	0.37 ± 0.06 ^{a, b}

Each femur was subjected to micro-CT at two ROIs—cortical (mid-diaphysis) and trabecular (distal metaphysis). With each bone, ten cortical parameters and eight trabecular parameters were assessed. Values are means and standard deviations. Statistical significance was determined by ANOVA followed by paired group comparisons. “a” and “b” are placed on the HFWD+minerals group: “a” shows statistically significant improvement relative to the HFWD group, “b” shows statistically significant improvement relative to CONTROL; “c” is placed on the CONTROL+minerals group, and shows significant improvement relative to the CONTROL (p <0.05). Data are based on 10 female mice in each diet group at 5 months.

Supplement Table 5. Micro-CT analysis of cortical and trabecular regions of femora from **female** mice in four diet groups at **twelve** months

	CONTROL	CONTROL + minerals	HFWD	HFWD + minerals
Cortical				
Bone mineral content (mg)	4.1 ± 0.2	4.4 ± 0.4	4.1 ± 0.3	4.6 ± 0.3 ^{a, b}
Bone mineral density (mg/cc)	309 ± 36	302 ± 31	287 ± 30	322 ± 21
Tissue mineral density (mg/cc)	1129 ± 14	1119 ± 23	1060 ± 13	1105 ± 18 ^{a, b}
Mean thickness (mm)	0.210 ± 0.011	0.218 ± 0.019	0.199 ± 0.010	0.218 ± 0.014 ^a
Polar moment of inertia (mm ⁴)	0.152 ± 0.013	0.173 ± 0.017 ^c	0.190 ± 0.036	0.188 ± 0.015 ^b
Endosteal perimeter (mm)	3.8 ± 0.1	4.0 ± 0.2 ^c	4.2 ± 0.2	4.1 ± 0.2 ^b
Periosteal perimeter (mm)	5.0 ± 0.1	5.3 ± 0.1 ^c	5.5 ± 0.4	5.5 ± 0.2 ^b
Marrow area (mm ²)	1.01 ± 0.07	1.10 ± 0.11 ^c	1.28 ± 0.10	1.16 ± 0.09 ^{a, b}
Cross sectional area (mm ²)	0.87 ± 0.04	0.94 ± 0.08 ^c	0.91 ± 0.07	0.96 ± 0.06 ^b
Total area (mm ²)	1.88 ± 0.07	2.03 ± 0.10 ^c	2.19 ± 0.16	2.12 ± 0.10 ^b
Trabecular				
Bone mineral content (mg)	0.44 ± 0.07	0.53 ± 0.09 ^c	0.58 ± 0.04	0.64 ± 0.07 ^{a, b}
Bone mineral density (mg/cc)	131 ± 21	158 ± 35	146 ± 11	171 ± 18 ^{a, b}
Tissue mineral density (mg/cc)	598 ± 30	587 ± 22	564 ± 33	569 ± 22 ^b
Bone volume fraction (mm ³ /mm ³)	0.02 ± 0.01	0.05 ± 0.03 ^c	0.04 ± 0.01	0.06 ± 0.02 ^{a, b}
Surface to volume ratio (mm ² /mm ³)	60.1 ± 7.1	50.8 ± 4.8 ^c	53.8 ± 8.7	53.5 ± 5.0 ^b
Trabecular thickness (mm)	0.034 ± 0.004	0.040 ± 0.004 ^c	0.038 ± 0.005	0.038 ± 0.004 ^b
Trabecular number (1/mm)	0.52 ± 0.16	1.25 ± 0.67 ^c	1.01 ± 0.30	1.49 ± 0.47 ^{a, b}
Trabecular spacing (mm)	2.13 ± 0.84	1.08 ± 0.81 ^c	1.04 ± 0.36	0.69 ± 0.21 ^{a, b}

Each femur was subjected to micro-CT at two ROIs—cortical (mid-diaphysis) and trabecular (distal metaphysis). With each bone, ten cortical parameters and eight trabecular parameters were assessed. Values are means and standard deviations. Statistical significance was determined by ANOVA followed by paired group comparisons. “a” and “b” are placed on the HFWD+minerals group: “a” shows statistically significant improvement relative to the HFWD group, “b” shows statistically significant improvement relative to CONTROL; “c” is placed on the CONTROL+minerals group, and shows significant improvement relative to the CONTROL (p <0.05). Data are based on 10 female mice in each diet group at 12 months.

Supplement Table 6. Micro-CT analysis of cortical and trabecular regions of femora from **female** mice in four diet groups at **eighteen** months

	CONTROL	CONTROL + minerals	HFWD	HFWD + minerals
Cortical				
Bone mineral content (mg)	4.0 ± 0.4	4.4 ± 0.5 ^c	3.6 ± 0.4	4.5 ± 0.6 ^{a, b}
Bone mineral density (mg/cc)	303 ± 27	299 ± 28	260 ± 34	322 ± 47 ^a
Tissue mineral density (mg/cc)	1105 ± 25	1132 ± 29 ^c	1076 ± 33	1140 ± 41 ^{a, b}
Mean thickness (mm)	0.201 ± 0.034	0.191 ± 0.017	0.159 ± 0.014	0.205 ± 0.022 ^a
Polar moment of inertia (mm ⁴)	0.181 ± 0.019	0.204 ± 0.030 ^c	0.195 ± 0.030	0.213 ± 0.029 ^b
Endosteal perimeter (mm)	4.1 ± 0.2	4.4 ± 0.1 ^c	4.7 ± 0.2	4.4 ± 0.2 ^{a, b}
Periosteal perimeter (mm)	5.4 ± 0.2	5.5 ± 0.2 ^c	5.7 ± 0.2	5.6 ± 0.2 ^b
Marrow area (mm ²)	1.20 ± 0.17	1.37 ± 0.11 ^c	1.63 ± 0.14	1.40 ± 0.16 ^{a, b}
Cross sectional area (mm ²)	0.88 ± 0.09	0.89 ± 0.08	0.79 ± 0.08	0.93 ± 0.13 ^a
Total area (mm ²)	2.08 ± 0.12	2.26 ± 0.13 ^c	2.42 ± 0.18	2.33 ± 0.14 ^b
Trabecular				
Bone mineral content (mg)	0.43 ± 0.13	0.50 ± 0.11	0.34 ± 0.14	0.44 ± 0.09 ^a
Bone mineral density (mg/cc)	120 ± 38	142 ± 40	79 ± 29	143 ± 30 ^a
Tissue mineral density (mg/cc)	556 ± 70	645 ± 24 ^c	604 ± 38	610 ± 40 ^b
Bone volume fraction (mm ³ /mm ³)	0.01 ± 0.01	0.04 ± 0.03 ^c	0.02 ± 0.01	0.03 ± 0.02 ^b
Surface to volume ratio (mm ² /mm ³)	62.2 ± 15.7	47.6 ± 7.1 ^c	57.8 ± 10.2	54.7 ± 8.9
Trabecular thickness (mm)	0.033 ± 0.010	0.043 ± 0.007 ^c	0.036 ± 0.008	0.038 ± 0.008
Trabecular number (1/mm)	0.33 ± 0.24	0.93 ± 0.73 ^c	0.52 ± 0.27	0.85 ± 0.73 ^b
Trabecular spacing (mm)	2.62 ± 1.25	1.70 ± 1.21	3.23 ± 3.27	1.42 ± 0.59 ^{a, b}

Each femur was subjected to micro-CT at two ROIs—cortical (mid-diaphysis) and trabecular (distal metaphysis). With each bone, ten cortical parameters and eight trabecular parameters were assessed. Values are means and standard deviations. Statistical significance was determined by ANOVA followed by paired group comparisons. “a” and “b” are placed on the HFWD+minerals group: “a” shows statistically significant improvement relative to the HFWD group, “b” shows statistically significant improvement relative to CONTROL; “c” is placed on the CONTROL+minerals group, and shows significant improvement relative to the CONTROL (p < 0.05). Data are based on 15 female mice in each diet group at 18 months.

Supplement Table 7. Micro-CT analysis of cortical and trabecular regions of C8 vertebrae from **female** mice in four diet groups at **twelve** months

	CONTROL	CONTROL + minerals	HFWD	HFWD + minerals
Cortical				
Bone mineral content (mg)	0.44 ± 0.05	0.43 ± 0.03	0.34 ± 0.03	0.43 ± 0.03 ^a
Bone mineral density (mg/cc)	961 ± 30	942 ± 14	731 ± 25	941 ± 33 ^a
Tissue mineral content (mg)	0.41 ± 0.05	0.41 ± 0.03	0.30 ± 0.03	0.40 ± 0.03 ^a
Tissue mineral density (mg/cc)	1056 ± 23	1050 ± 15	962 ± 13	1044 ± 25 ^a
Mean thickness (mm)	0.31 ± 0.04	0.27 ± 0.01	0.22 ± 0.02	0.28 ± 0.04 ^a
Inner perimeter (mm)	2.38 ± 0.28	2.86 ± 0.14 ^c	2.68 ± 0.14	2.70 ± 0.33
Outer perimeter (mm)	5.05 ± 0.38	5.31 ± 0.22	5.22 ± 0.31	5.28 ± 0.28
Marrow area (mm ²)	0.39 ± 0.09	0.52 ± 0.04 ^c	0.51 ± 0.06	0.48 ± 0.12
Cortical area (mm ²)	0.97 ± 0.11	0.92 ± 0.06	0.73 ± 0.06	0.95 ± 0.09 ^a
Total Area (mm ²)	1.36 ± 0.09	1.45 ± 0.09	1.24 ± 0.05	1.43 ± 0.06 ^a
Volume (mm ³)	0.46 ± 0.05	0.46 ± 0.02	0.47 ± 0.03	0.46 ± 0.02
Volume of bone (mm ³)	0.39 ± 0.05	0.39 ± 0.02	0.31 ± 0.02	0.38 ± 0.02 ^a
Trabecular				
Bone mineral content (mg)	0.40 ± 0.07	0.55 ± 0.08 ^c	0.30 ± 0.03	0.40 ± 0.06 ^a
Bone mineral density (mg/cc)	619 ± 63	737 ± 83 ^c	482 ± 34	687 ± 107 ^a
Tissue mineral content (mg)	0.36 ± 0.08	0.51 ± 0.09 ^c	0.25 ± 0.03	0.38 ± 0.07 ^a
Tissue mineral density (mg/cc)	808 ± 28	858 ± 49	711 ± 15	794 ± 45 ^a
Bone volume fraction (mm ³ /mm ³)	0.67 ± 0.07	0.79 ± 0.08 ^c	0.56 ± 0.05	0.80 ± 0.14 ^a
Surface to volume ratio (mm ² /mm ³)	17.3 ± 2.7	13.1 ± 3.3 ^c	20.1 ± 3.0	14.8 ± 6.3
Trabecular thickness (mm)	0.12 ± 0.02	0.16 ± 0.04 ^c	0.10 ± 0.02	0.15 ± 0.05 ^a
Trabecular number (1/mm)	5.7 ± 0.3	5.1 ± 0.8	5.6 ± 0.8	5.5 ± 1.1
Trabecular spacing (mm)	0.06 ± 0.01	0.04 ± 0.01 ^c	0.08 ± 0.02	0.03 ± 0.02 ^{a, b}
Volume (mm ³)	0.65 ± 0.07	0.75 ± 0.05 ^c	0.62 ± 0.03	0.59 ± 0.04
Volume of bone (mm ³)	0.44 ± 0.08	0.60 ± 0.07 ^c	0.35 ± 0.04	0.47 ± 0.07 ^a

Each C8 vertebra (from a subset) was subjected to micro-CT at two ROIs—cortical (middle isosurface) and trabecular (cranial isosurface). With each vertebra, twelve cortical parameters and eleven trabecular parameters were assessed. Values are means and standard deviations. Statistical significance was determined by ANOVA followed by paired group comparisons. “a” and “b” are placed on the HFWD+minerals group: “a” shows statistically significant improvement relative to the HFWD group, “b” shows statistically significant improvement relative to CONTROL; “c” is placed on the CONTROL+minerals group, and shows significant improvement relative to the CONTROL ($p < 0.05$). Vertebral data are based on 6 female mice at 12 months in each diet group.

Supplement Table 8. Micro-CT analysis of cortical and trabecular regions of C8 vertebrae from **female** mice in two diet groups at **eighteen** months

	HFWD	HFWD + minerals
Cortical		
Bone mineral content (mg)	0.32 ± 0.03	0.39 ± 0.06 ^a
Bone mineral density (mg/cc)	763 ± 146	928 ± 43
Tissue mineral content (mg)	0.28 ± 0.03	0.36 ± 0.06 ^a
Tissue mineral density (mg/cc)	960 ± 74	1028 ± 47
Mean thickness (mm)	0.21 ± 0.04	0.25 ± 0.06
Inner perimeter (mm)	2.78 ± 0.31	2.48 ± 0.24
Outer perimeter (mm)	4.91 ± 0.25	4.90 ± 0.41
Marrow area (mm ²)	0.54 ± 0.14	0.41 ± 0.06
Cortical area (mm ²)	0.62 ± 0.04	0.86 ± 0.10 ^a
Total area (mm ²)	1.16 ± 0.15	1.27 ± 0.12
Volume (mm ³)	0.43 ± 0.07	0.41 ± 0.05
Volume of bone (mm ³)	0.29 ± 0.02	0.35 ± 0.04 ^a
Trabecular		
Bone mineral content (mg)	0.32 ± 0.04	0.42 ± 0.07
Bone mineral density (mg/cc)	452 ± 46	659 ± 108 ^a
Tissue mineral content (mg)	0.25 ± 0.04	0.37 ± 0.07 ^a
Tissue mineral density (mg/cc)	768 ± 46	846 ± 58
Bone volume fraction (mm ³ /mm ³)	0.47 ± 0.05	0.69 ± 0.10 ^a
Surface to volume ratio (mm ² /mm ³)	21.9 ± 2.7	15.1 ± 3.4 ^a
Trabecular thickness (mm)	0.09 ± 0.01	0.14 ± 0.03 ^a
Trabecular number (1/mm)	5.0 ± 0.3	5.0 ± 0.5
Trabecular spacing (mm)	0.11 ± 0.01	0.06 ± 0.02 ^a
Volume (mm ³)	0.70 ± 0.05	0.64 ± 0.07
Volume of bone (mm ³)	0.33 ± 0.04	0.44 ± 0.07

Each C8 vertebra (from a subset) was subjected to micro-CT at two ROIs—cortical (middle isosurface) and trabecular (cranial isosurface). With each vertebra, twelve cortical parameters and eleven trabecular parameters were assessed. Values are means and standard deviation. Statistical significance was determined by paired group comparisons (p<0.05). “a” is placed on the HFWD+minerals group: “a” shows statistically significant improvement relative to the HFWD group. Vertebral data are based on 6 female mice each of the two high-fat diets at 18 months.

**Supplement
Table 9A.**

**Bone Mineral Analysis at 5 months in long
bones of female mice($\mu\text{g/g}$)**

5 months

	CONTROL	CONTROL+ minerals	HFWD	HFWD+ minerals
Fluoride	0.83	1.06	0.85	0.88
Barium	4.32	5.2	7.58	3.4
Boron	<0.5	<0.5	<0.5	<0.5
Calcium	207400	219900	182600	209100
Copper	<0.5	<0.5	<0.5	<0.5
Iron	105	81	315	100
Lanthanum	0.99	0.75	1.2	0.66
Magnesium	3232	3425	2891	3250
Manganese	1.93	1.77	6.3	1.56
Phosphorus	99597	105564	88548	98629
Potassium	856	907	832	982
Selenium	<0.5	<0.5	<0.5	<0.5
Silicon	3.1	<0.5	2.91	<0.5
Strontium	54.6	261	126	235
Zinc	129	151	246	149

The long bones (one femur and tibia from each animal in the group) were “pooled” and analyzed for levels of trace metals found in the mineral-rich product to give a single value at each time point. Some of these elements were recorded below detectable levels when their concentration level found below $0.5\mu\text{g/g}$. The levels of individual trace elements were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) except Fluoride which was done by AOAC 984.37 assay.

Supplement Table
9 B

Bone Mineral Analysis at 12 and 18 months in long bones of female mice (µg/g)

	<u>12 months</u>				<u>18 months</u>			
	CONTROL	CONTROL+ minerals	HFWD	HFWD+ minerals	CONTROL	CONTROL+ minerals	HFWD	HFWD+ minerals
Fluoride	6.1	8.31	4.9	5.86	6.35	7.06	6.83	6.13
Aluminum	0.98	3.46	2.04	2.79	<0.5	<0.5	<0.5	<0.5
Antimony	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Arsenic	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Barium	3.84	5.43	5.21	3.03	3.06	3.92	4.45	2.41
Beryllium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Bismuth	<0.5	<0.5	2.62	<0.5	<0.5	<0.5	<0.5	<0.5
Boron	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Calcium	226800	234900	215900	232800	221400	230300	202500	222400
Cadmium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Chromium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Cobalt	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Copper	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Iron	213	120	374	113	107	126	301	85.2
Lanthanum	<0.5	<0.5	0.58	<0.5	<0.5	<0.5	<0.5	<0.5
Lead	<0.5	<0.5	<0.5	<0.5	<0.5	0.95	1.39	1.03
Lithium	0.52	0.89	<0.5	0.63	1.98	1.35	0.83	0.78
Magnesium	3386	3671	3258	3803	3712	4122	3324	4010
Manganese	1.47	1.46	3.95	1.89	1.58	1.29	3.47	1.24
Mercury	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Molybdenum	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Nickel	<0.5	0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Niobium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Phosphorus	118400	119800	110400	116300	105500	112200	97980	108400
Potassium	897	977	1044	1153	1279	1342	1295	1595
Selenium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Silicon	0.57	<0.5	0.57	<0.5	0.71	<0.5	0.61	<0.5
Silver	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Sodium	11890	11220	11780	12500	15090	15940	14460	16200
Strontium	27.31	282	52.3	256	24.48	255	46.64	230
Sulfur	3297	3228	3237	3398	3153	3175	2917	3115
Tellurium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Thallium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Thorium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Tin	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Titanium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Tungsten	<0.5	0.566	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Vanadium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Yttrium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Zinc	157	197	246	191	150	174	219	174
Zirconium	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

The long bones (one femur and tibia from each animal in the group) were “pooled” and analyzed for levels of trace metals found in the mineral-rich product to give a single value at each time point. Some of these elements were recorded below detectable levels when their concentration level found below 0.5µg/g. The levels of individual trace elements were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) except Fluoride which was done by AOAC 984.37 assay.