QC Reviewed By Jackie O'Brien 17 NOV 2005

Study No.: SC00204

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Treatment A: Aquamin F 600 mg (200 mg TID)

Treatment B: Calcium Carbonate 600 mg (200 mg TID)

Treatment C: Tricalcium Phosphate 600 mg (200 mg TID)

Treatment D: Placebo

Introduction

The level of two bone markers in the volunteers' urine (Urine, DPD adjusted for Creatinne) and four bone markers in the volunteers' serum (BAP, PTH, Vitamin D, Osteocalcin) was measured before the volunteers began taking one of four treatments. The treatments are Treatment A (Aquamin F 600 mg, 200 mg TID), Treatment B (Calcium Carbonate 600 mg, 200 mg TID), Treatment C (Tricalcium Phosphate 600 mg, 200 mg TID) and Treatment D (Placebo). At the end of the trial the level of each bone marker in the urine was measured a second time. The purpose of this analysis is to assess the change in the level of each type of bone marker in the urine at the end of the trial. To determine this the pre dose levels of the bone marker was subtracted from the post-dose levels of the bone marker. The difference in the amount of bone marker is the measure of the effect of taking the treatment. A total of 21 volunteers completed the trial.

Summary statistics

The summary statistics (mean, SD, minimum, maximum and median) for the difference in the level of each of the six bone markers of interest are shown in Table 1. See Tables A-1 to A-3 of this report for further details.

Table 1: Summary statistics for the computed pharmacokinetic parameters

Statistics	BAP	PTH	Vitamin D	Osteocalcin	DPD	Calcium
Mean	1.44	-0.39	1.23	-0.32	-0.40	0.39
SD	9.61	0.73	20.20	1.80	2.19	1.42
Minimum	-12.78	-1.85	-32.85	-3.86	-6.12	-2.56
Maximum	30.85	1.08	36.09	2.31	3.71	4.02
Median	2.40	-0.62	1.88	-0.37	-0.17	0.11

This information is presented for BAP in skeletal box plots in Figure 1. Similar graphs have been provided in Table A-6 for the other markers of interest.

Figure 1: Box plot of the change in BAP at the end of the trial

Boxplot of the change in BAP at the end of the trial according to treatment
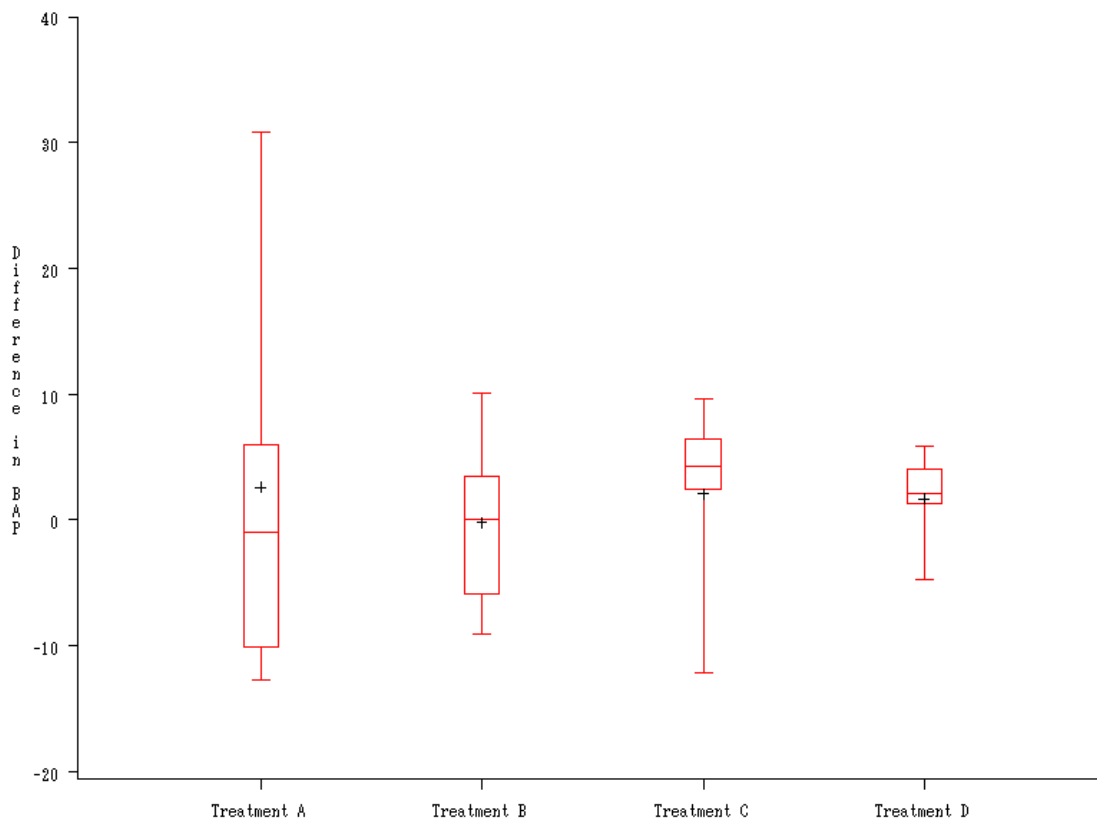
Statistical Analysis of Data

To assess how the concentration of the markers changed the data for the difference in the level of bone markers was subjected to analyses of variance (ANOVA) using SAS® PROC GLM. The ANOVA model used for each of the markers was:

$$Marker \sim \beta_0 + \beta_1 Treatment_{in} + \varepsilon_{ijkmn}$$

Marker
This is the one of the following markers: BAP, PTH, Vitamin D, Osteocalcin and DPD. It is sometimes called the dependent variable or the response. The manner in which Marker depends on Treatment is determined by β_0 and β_1 (the regression coefficients).

β_0
The coefficient β_0 is called the intercept and it is the expected value of Marker for Treatment D.

β_1
The coefficient β_1 measures the difference between the mean for any of the treatments and the mean for Treatment D.

Treatment
This predictor refers to which drug was consumed by the ith volunteer. When j is 1 we are referring to Treatment A, when it is 2 we are referring to Treatment B, when it is 3 we are referring to Treatment C and when it is 4 we are referring to Treatment D.

Type II sums of squares

The type two sums of squares provide an overall measure of how important Treatment is in determining bone marker. P-values from this part of the ANOVA analysis are presented in Table 2. None of the p-values indicate a statistically significant treatment effect. Further information on the results of the ANOVA analysis is contained in Tables B-1 to B-6 of this report.

Table 2: Summary of the ANOVA model

	BAP	PTH	Vitamin D	Osteocalcin	DPD	Calcium
Between treatments p-value	0.9699	0.1885	0.3198	0.3098	0.3411	0.1760

Comparison of interest

In this study it was of particular interest to compare treatment A to the other three treatments to see if there was a statistically significant difference in the means. The following table provides p-values from these comparisons. In cases where there was a statistically significant difference the p-value has been highlighted in the table. See Tables B-1 to B-6 for further details.

Table 3: Summary of the ANOVA model

Bone Marker	A vs. B	A vs. C	A vs. D
BAP	0.6581	0.9393	0.8921
PTH	0.3010	0.1800	0.0359
Vitamin D	0.9897	0.4557	0.2631
Osteocalcin	0.1642	0.6850	0.7151
DPD	0.1380	0.1156	0.1727
Calcium	0.6904	0.3206	0.2279

As there was a statistically significant difference between Treatments A and D for PTH the difference in means for B vs. C, B vs. D and C vs. D was examined. There were no statistically significant differences between these treatments. See Table B-2 for further details.
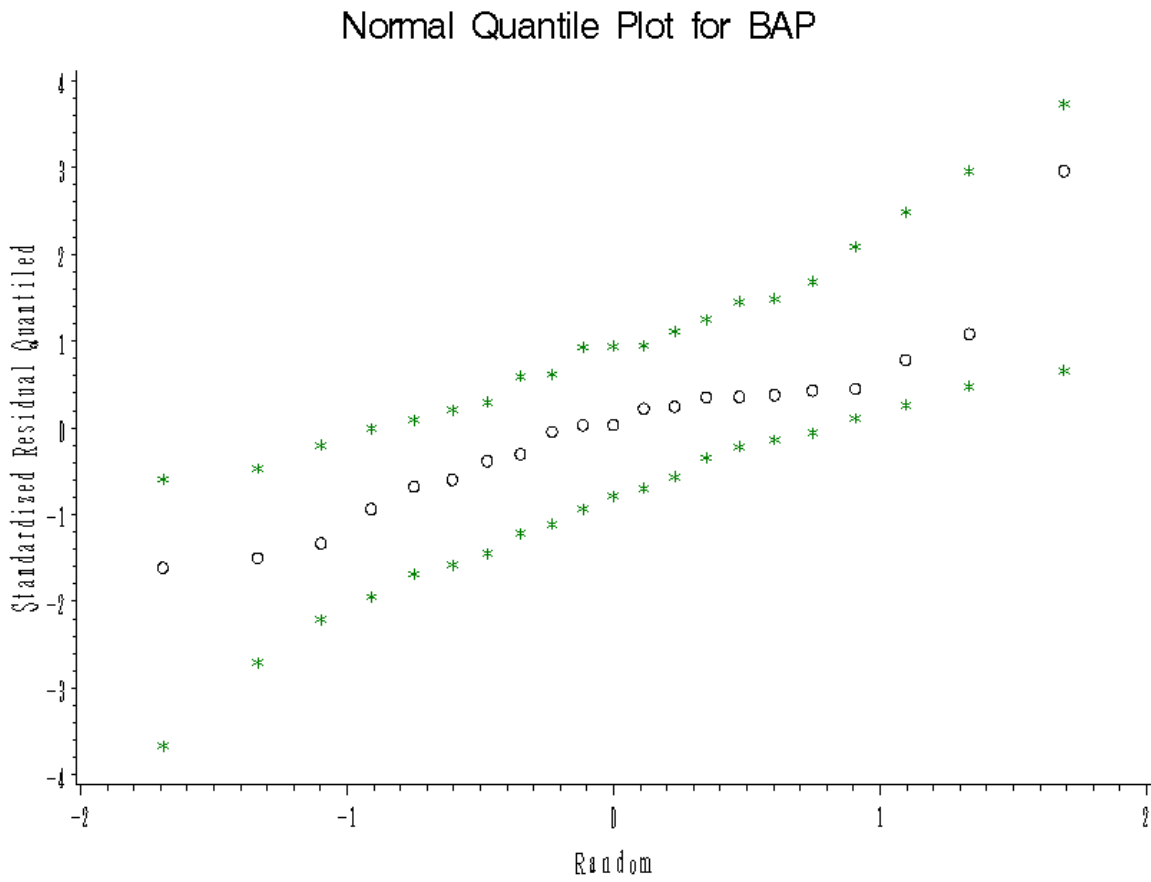
Statistical outliers and exclusions

Exclusion of data from the analyses was considered on the basis of discordant values (outlier data) for specific pharmacokinetic parameters revealed by the Studentized residual (SR), using Lund's value of 2.72 as a critical SR value for exclusion. Volunteer 6 was a statistical outlier for BAP but was not removed from the analysis. Studentized residual (SR) values are tabulated in Table C-1 of this report.

Assumptions underlying the ANOVA model

An ANOVA model relies on the assumption that each data set was from a Gaussian distribution with a characteristic mean and standard deviation. To assess this assumption a qq-plot comparing the residuals to a standard normal distribution was made for each pharmacokinetic parameter. A qq- (or normal probability) plot shows the ordered statistics for the observed data set plotted against the ordered statistics for the normal data. If the observed plot of the data is approximately linear then the assumption of normality is satisfied. The observed data is represented by 'o'. To help the assessment of normality 95% upper and lower bounds were created around the observed data. If the assumption of normality was correct then 95% of the time samples from a standard normal distribution should lie in the area bounded by the '*''s. The 95% upper and lower bounds for the order statistics were created using two data sets of 21 points drawn from 1,000 data sets each containing 21 random numbers from N(0,1). Figure 2 shows the qq-plot for BAP. As can be seen from the graph the residuals appear to be normally distributed and the assumptions underlying the ANOVA model satisfied. The graphs for the other markers may be seen in Table D-1 of this report.

Figure 2: qq-plot of the residuals from the ANOVA model for BAP

Normal Quantile Plot for BAP

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Table A- 1: Summary of bone marker data

Subject	Treatment	BAP			PTH		
		Pre Dose	Post Dose	Difference	Pre Dose	Post Dose	Difference
1	D	32.53	34.56	2.03	0.89	0.69	-0.20
2	D	21.68	22.96	1.28	1.51	2.28	0.77
3	B	29.01	23.09	-5.92	1.34	1.37	0.02
4	A	31.10	30.05	-1.05	2.88	1.03	-1.85
5	C	20.79	25.01	4.22	3.70	3.07	-0.63
6	A	85.48	116.34	30.85	2.62	1.89	-0.73
7	D	51.14	56.93	5.80	1.60	0.92	-0.68
8	C	20.23	26.62	6.39	3.56	2.89	-0.67
9	B	29.72	32.92	3.20	4.44	3.82	-0.62
10	C	28.18	30.58	2.40	2.95	1.79	-1.16
11	A	29.98	35.95	5.97	2.56	1.86	-0.70
12	B	29.92	33.31	3.39	1.53	0.63	-0.90
13	C	34.30	43.87	9.57	0.85	1.93	1.08
14	B	29.10	39.21	10.11	2.76	2.62	-0.14
15	C	36.14	23.92	-12.22	3.73	3.66	-0.06
16	D	23.85	19.08	-4.77	1.65	2.16	0.51
17	A	30.77	20.66	-10.10	1.89	1.40	-0.49
18	B	36.60	27.46	-9.15	2.37	0.83	-1.54
19	A	50.05	37.26	-12.78	3.17	2.47	-0.70
20	B	21.75	18.64	-3.11	3.46	3.93	0.47
21	D	23.28	27.33	4.05	0.61	0.70	0.08
Number		21	21	21	21	21	21
Mean		33.12	34.56	1.44	2.38	2.00	-0.39
Standard Deviation		14.55	20.82	9.61	1.08	1.05	0.73
Minimum		20.23	18.64	-12.78	0.61	0.63	-1.85
Maximum		85.48	116.34	30.85	4.44	3.93	1.08
Median		29.92	30.05	2.40	2.56	1.89	-0.62

Table A- 2: Summary of bone marker data

Subject	Treatment	Vitamin D			Osteocalcin		
		Pre Dose	Post Dose	Difference	Pre Dose	Post Dose	Difference
1	D	74.14	107.04	32.89	11.57	12.16	0.59
2	D	62.07	90.40	28.33	9.22	9.74	0.52
3	B	77.35	61.44	-15.90	8.80	5.77	-3.04
4	A	58.37	50.88	-7.50	9.69	11.35	1.67
5	C	56.50	58.38	1.88	9.64	10.35	0.71
6	A	48.46	50.51	2.04	13.49	12.48	-1.01
7	D	66.37	98.19	31.82	13.91	16.22	2.31
8	C	59.41	30.62	-28.79	6.67	6.93	0.27
9	B	54.75	66.64	11.90	12.23	9.97	-2.26
10	C	71.31	56.39	-14.92	17.04	13.18	-3.86
11	A	41.91	78.00	36.09	15.09	14.20	-0.88
12	B	90.99	102.29	11.30	8.35	9.87	1.52
13	C	82.01	79.82	-2.19	11.17	13.47	2.29
14	B	56.51	71.23	14.72	11.20	10.83	-0.37
15	C	40.00	36.65	-3.35	11.33	10.28	-1.06
16	D	35.48	41.77	6.30	10.19	9.37	-0.82
17	A	44.52	47.22	2.69	11.15	10.52	-0.63
18	B	48.50	31.98	-16.53	9.55	5.82	-3.74
19	A	103.45	70.60	-32.85	15.36	16.86	1.50
20	B	65.40	59.55	-5.85	7.27	6.63	-0.64
21	D	99.08	72.78	-26.30	9.19	9.31	0.12
Number		21	21	21	21	21	21
Mean		63.65	64.87	1.23	11.05	10.73	-0.32
Standard Deviation		18.94	22.28	20.20	2.70	3.04	1.80
Minimum		35.48	30.62	-32.85	6.67	5.77	-3.86
Maximum		103.45	107.04	36.09	17.04	16.86	2.31
Median		59.41	61.44	1.88	11.15	10.35	-0.37

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Table A- 3: Summary of bone marker data

Subject	Treatment	DPD			Calcium (Total)		
		Pre Dose	Post Dose	Difference	Pre Dose	Post Dose	Difference
1	D	7.33	9.08	1.75	2.20	6.23	4.02
2	D	9.68	7.06	-2.62	0.00	1.89	1.89
3	B	8.57	6.29	-2.28	0.00	0.85	0.85
4	A	6.67	8.85	2.18	3.20	2.32	-0.88
5	C	7.71	7.75	0.04	3.40	2.93	-0.48
6	A	12.28	9.45	-2.83	4.00	3.10	-0.90
7	D	8.88	8.35	-0.53	2.95	3.06	0.11
8	C	5.49	6.80	1.31	1.32	1.01	-0.31
9	B	4.89	5.99	1.10	0.00	1.61	1.61
10	C	9.33	9.90	0.57	4.06	2.70	-1.36
11	A	12.45	6.33	-6.12	0.00	2.21	2.21
12	B	8.38	12.09	3.71	3.36	3.52	0.16
13	C	13.12	13.10	-0.02	0.00	0.00	0.00
14	B	9.43	8.33	-1.10	2.56	0.00	-2.56
15	C	6.76	6.28	-0.48	0.00	0.00	0.00
16	D	8.05	7.88	-0.17	0.00	0.00	0.00
17	A	5.91	5.44	-0.47	0.00	0.00	0.00
18	B	7.54	5.40	-2.14	1.92	2.10	0.18
19	A	8.89	6.25	-2.64	2.18	3.94	1.76
20	B	5.95	6.99	1.04	1.75	2.17	0.42
21	D	5.43	6.82	1.39	2.94	4.42	1.48
Number		21	21	21	21	21	21
Mean		8.23	7.83	-0.40	1.71	2.10	0.39
Standard Deviation		2.31	2.05	2.19	1.52	1.67	1.42
Minimum		4.89	5.40	-6.12	0.00	0.00	-2.56
Maximum		13.12	13.10	3.71	4.06	6.23	4.02
Median		8.05	7.06	-0.17	1.92	2.17	0.11

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Table A- 4: Data listing of plasma concentration data

SCO0204: Osteoporosis Study
Treatment A= Aquamin F 600 mg (200 mg TID)
Treatment B= Calcium Carbonate 600 mg (200 mg TID)
Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
Treatment D= Placebo
Copy of the Osteoporosis data set

Subject	Treatment	BAP_Post	BAP_Pre	BAP	PTH_Post	PTH_Pre	PTH	VitD_Post	VitD_Pre	VitD
1	D	34.560	32.530	2.030	0.685	0.888	-0.203	107.037	74.144	32.893
2	D	22.959	21.682	1.277	2.283	1.510	0.773	90.399	62.071	28.328
3	B	23.087	29.009	-5.922	1.365	1.341	0.024	61.442	77.346	-15.904
4	A	30.048	31.097	-1.049	1.030	2.884	-1.854	50.876	58.373	-7.497
5	C	25.010	20.791	4.219	3.072	3.701	-0.629	58.377	56.496	1.881
6	A	116.338	85.484	30.854	1.885	2.615	-0.730	50.506	48.462	2.044
7	D	56.934	51.138	5.796	0.920	1.602	-0.682	98.191	66.374	31.817
8	C	26.620	20.230	6.390	2.892	3.557	-0.665	30.616	59.408	-28.792
9	B	32.920	29.724	3.196	3.820	4.435	-0.615	66.640	54.745	11.895
10	C	30.581	28.184	2.397	1.790	2.947	-1.157	56.387	71.308	-14.921
11	A	35.948	29.983	5.965	1.857	2.558	-0.701	77.998	41.906	36.092
12	B	33.312	29.918	3.394	0.632	1.528	-0.896	102.290	90.990	11.300
13	C	43.868	34.297	9.571	1.929	0.847	1.082	79.820	82.007	-2.187
14	B	39.208	29.096	10.112	2.619	2.762	-0.143	71.234	56.510	14.724
15	C	23.918	36.140	-12.222	3.664	3.727	-0.063	36.653	40.002	-3.349
16	D	19.080	23.854	-4.774	2.163	1.651	0.512	41.774	35.475	6.299
17	A	20.664	30.765	-10.101	1.400	1.885	-0.485	47.215	44.522	2.693
18	B	27.456	36.603	-9.147	0.830	2.372	-1.542	31.977	48.502	-16.525
19	A	37.263	50.047	-12.784	2.467	3.170	-0.703	70.603	103.450	-32.847
20	B	18.635	21.746	-3.111	3.926	3.456	0.470	59.550	65.400	-5.850
21	D	27.327	23.278	4.049	0.695	0.614	0.081	72.777	99.076	-26.299

| Subject	Treatment	Osteocalcin_Post	Osteocalcin_Pre	Osteocalcin	DPD_Post	DPD_Pre	DPD
1	D	12.161	11.570	0.591	9.08	7.33	1.75
2	D	9.742	9.224	0.518	7.06	9.68	-2.62
3	B	5.767	8.803	-3.036	6.29	8.57	-2.28
4	A	11.354	9.688	1.666	8.85	6.67	2.18
5	C	10.345	9.639	0.706	7.75	7.71	0.04
6	A	12.480	13.489	-1.009	9.45	12.28	-2.83
7	D	16.219	13.913	2.306	8.35	8.88	-0.53
8	C	6.934	6.665	0.269	6.80	5.49	1.31
9	B	9.969	12.231	-2.262	5.99	4.89	1.10
10	C	13.176	17.036	-3.860	9.90	9.33	0.57
11	A	14.203	15.086	-0.883	6.33	12.45	-6.12
12	B	9.870	8.350	1.520	12.09	8.38	3.71
13	C	13.466	11.173	2.293	13.10	13.12	-0.02
14	B	10.831	11.196	-0.365	8.33	9.43	-1.10
15	C	10.279	11.334	-1.055	6.28	6.76	-0.48
16	D	9.367	10.186	-0.819	7.88	8.05	-0.17
17	A	10.517	11.145	-0.628	5.44	5.91	-0.47
18	B	5.815	9.550	-3.735	5.40	7.54	-2.14
19	A	16.864	15.361	1.503	6.25	8.89	-2.64
20	B	6.631	7.269	-0.638	6.99	5.95	1.04
21	D	9.308	9.190	0.118	6.82	5.43	1.39

Study No. SC00204. Report.

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

SC00204: Osteoporosis Study
Treatment A= Aquamin F 600 mg (200 mg TID)
Treatment B= Calcium Carbonate 600 mg (200 mg TID)
Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
Treatment D= Placebo
Copy of the Osteoporosis data set

Subject	Treatment	Pre_9	Calcium_ Pre	Calcium_ Pre_Total	Post_9	Calcium_ Post	Calcium_ Post_ Total	Calcium
1	D	760	2.9	2.2040	1415	4.4	6.2260	4.0220
2	D	2440	0.0	0.0000	900	2.1	1.8900	1.8900
3	B	1590	0.0	0.0000	370	2.3	0.8510	0.8510
4	A	640	5.0	3.2000	580	4.0	2.3200	-0.8800
5	C	1700	2.0	3.4000	1170	2.5	2.9250	-0.4750
6	A	1380	2.9	4.0020	970	3.2	3.1040	-0.8980
7	D	1340	2.2	2.9480	485	6.3	3.0555	0.1075
8	C	630	2.1	1.3230	505	2.0	1.0100	-0.3130
9	B	1462	0.0	0.0000	670	2.4	1.6080	1.6080
10	C	2135	1.9	4.0565	1500	1.8	2.7000	-1.3565
11	A	1890	0.0	0.0000	1700	1.3	2.2100	2.2100
12	B	2240	1.5	3.3600	2200	1.6	3.5200	0.1600
13	C	2220	0.0	0.0000	2700	0.0	0.0000	0.0000
14	B	1420	1.8	2.5560	1990	0.0	0.0000	-2.5560
15	C	1490	0.0	0.0000	2370	0.0	0.0000	0.0000
16	D	2050	0.0	0.0000	2740	0.0	0.0000	0.0000
17	A	2800	0.0	0.0000	3440	0.0	0.0000	0.0000
18	B	600	3.2	1.9200	840	2.5	2.1000	0.1800
19	A	750	2.9	2.1750	1640	2.4	3.9360	1.7610
20	B	1030	1.7	1.7510	1140	1.9	2.1660	0.4150
21	D	840	3.5	2.9400	1380	3.2	4.4160	1.4760

Table A- 5: Summary of plasma concentration data

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                  SC00204: Osteoporosis Study
               Treatment A= Aquamin F 600 mg (200 mg TID)
            Treatment B= Calcium Carbonate 600 mg (200 mg TID)
         Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                       Treatment D= Placebo
               Summary Statistics for Osteoporosis

                        The MEANS Procedure
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N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum	Median	Coeff of Variation
21	BAP_Post	BAP_Post	21	34.56	20.82	18.64	116.34	30.05	60.24
	BAP_Pre	BAP_Pre	21	33.12	14.55	20.23	85.48	29.92	43.94
	BAP		21	1.44	9.61	-12.78	30.85	2.40	669.59
	PTH_Post	PTH_Post	21	2.00	1.05	0.63	3.93	1.89	52.62
	PTH_Pre	PTH_Pre	21	2.38	1.08	0.61	4.44	2.56	45.38
	PTH		21	-0.39	0.73	-1.85	1.08	-0.62	-187.88
	VitD_Post	VitD_Post	21	64.87	22.28	30.62	107.04	61.44	34.34
	VitD_Pre	VitD_Pre	21	63.65	18.94	35.48	103.45	59.41	29.75
	VitD		21	1.23	20.20	-32.85	36.09	1.88	1644.72
	Osteocalcin_Post	Osteocalcin_Post	21	10.73	3.04	5.77	16.86	10.35	28.38
	Osteocalcin_Pre	Osteocalcin_Pre	21	11.05	2.70	6.67	17.04	11.15	24.46
	Osteocalcin		21	-0.32	1.80	-3.86	2.31	-0.37	-555.11
	DPD_Post	DPD_Post	21	7.83	2.05	5.40	13.10	7.06	26.15
	DPD_Pre	DPD_Pre	21	8.23	2.31	4.89	13.12	8.05	28.14
	DPD		21	-0.40	2.19	-6.12	3.71	-0.17	-552.51
	Calcium_Pre	Calcium_Pre	21	1.60	1.49	0.00	5.00	1.80	92.91
	Calcium_Pre_Total		21	1.71	1.52	0.00	4.06	1.92	89.23
	Calcium_Post	Calcium_Post	21	2.09	1.62	0.00	6.30	2.10	77.56
	Calcium_Post_Total		21	2.10	1.67	0.00	6.23	2.17	79.71
	Calcium		21	0.39	1.42	-2.56	4.02	0.11	364.52

Treatment	N Obs	Variable	Label	N	Mean	Std Dev	Minimum	Maximum	Median
A	5	BAP_Post	BAP_Post	5	48.05	38.73	20.66	116.34	35.95
		BAP_Pre	BAP_Pre	5	45.48	23.90	29.98	85.48	31.10
		BAP		5	2.58	17.47	-12.78	30.85	-1.05
		PTH_Post	PTH_Post	5	1.73	0.54	1.03	2.47	1.86
		PTH_Pre	PTH_Pre	5	2.62	0.48	1.89	3.17	2.62
		PTH		5	-0.89	0.55	-1.85	-0.48	-0.70
		VitD_Post	VitD_Post	5	59.44	13.89	47.22	78.00	50.88
		VitD_Pre	VitD_Pre	5	59.34	25.44	41.91	103.45	48.46
		VitD		5	0.10	24.74	-32.85	36.09	2.04
		Osteocalcin_Post	Osteocalcin_Post	5	13.08	2.52	10.52	16.86	12.48
		Osteocalcin_Pre	Osteocalcin_Pre	5	12.95	2.48	9.69	15.36	13.49
		Osteocalcin		5	0.13	1.34	-1.01	1.67	-0.63
		DPD_Post	DPD_Post	5	7.26	1.77	5.44	9.45	6.33
		DPD_Pre	DPD_Pre	5	9.24	3.06	5.91	12.45	8.89
		DPD		5	-1.98	3.08	-6.12	2.18	-2.64
		Calcium_Pre	Calcium_Pre	5	2.16	2.15	0.00	5.00	2.90
		Calcium_Pre_Total		5	1.88	1.83	0.00	4.00	2.18
		Calcium_Post	Calcium_Post	5	2.18	1.58	0.00	4.00	2.40
		Calcium_Post_Total		5	2.31	1.47	0.00	3.94	2.32
		Calcium		5	0.44	1.47	-0.90	2.21	0.00
B	6	BAP_Post	BAP_Post	6	29.10	7.52	18.64	39.21	30.19
		BAP_Pre	BAP_Pre	6	29.35	4.71	21.75	36.60	29.41
		BAP		6	-0.25	7.10	-9.15	10.11	0.04
		PTH_Post	PTH_Post	6	2.20	1.47	0.63	3.93	1.99
		PTH_Pre	PTH_Pre	6	2.65	1.17	1.34	4.44	2.57
		PTH		6	-0.45	0.72	-1.54	0.47	-0.38
		VitD_Post	VitD_Post	6	65.52	22.64	31.98	102.29	64.04
		VitD_Pre	VitD_Pre	6	65.58	15.97	48.50	90.99	60.96
		VitD		6	-0.06	14.47	-16.53	14.72	2.73
		Osteocalcin_Post	Osteocalcin_Post	6	8.15	2.32	5.77	10.83	8.25
		Osteocalcin_Pre	Osteocalcin_Pre	6	9.57	1.85	7.27	12.23	9.18
		Osteocalcin		6	-1.42	1.95	-3.74	1.52	-1.45
		DPD_Post	DPD_Post	6	7.52	2.46	5.40	12.09	6.64
		DPD_Pre	DPD_Pre	6	7.46	1.72	4.89	9.43	7.96
		DPD		6	0.06	2.32	-2.28	3.71	-0.03
		Calcium_Pre	Calcium_Pre	6	1.37	1.22	0.00	3.20	1.60
		Calcium_Pre_Total		6	1.60	1.36	0.00	3.36	1.84
		Calcium_Post	Calcium_Post	6	1.78	0.94	0.00	2.50	2.10
		Calcium_Post_Total		6	1.71	1.21	0.00	3.52	1.85
		Calcium		6	0.11	1.41	-2.56	1.61	0.30
C	5	BAP_Post	BAP_Post	5	30.00	8.15	23.92	43.87	26.62
		BAP_Pre	BAP_Pre	5	27.93	7.39	20.23	36.14	28.18
		BAP		5	2.07	8.42	-12.22	9.57	4.22
		PTH_Post	PTH_Post	5	2.67	0.79	1.79	3.66	2.89
		PTH_Pre	PTH_Pre	5	2.96	1.22	0.85	3.73	3.56
		PTH		5	-0.29	0.86	-1.16	1.08	-0.63
		VitD_Post	VitD_Post	5	52.37	19.53	30.62	79.82	56.39
		VitD_Pre	VitD_Pre	5	61.84	15.87	40.00	82.01	59.41
		VitD		5	-9.47	12.47	-28.79	1.88	-3.35
		Osteocalcin_Post	Osteocalcin_Post	5	10.84	2.65	6.93	13.47	10.35
		Osteocalcin_Pre	Osteocalcin_Pre	5	11.17	3.78	6.67	17.04	11.17
		Osteocalcin		5	-0.33	2.31	-3.86	2.29	0.27
		DPD_Post	DPD_Post	5	8.77	2.79	6.28	13.10	7.75
		DPD_Pre	DPD_Pre	5	8.48	2.95	5.49	13.12	7.71
		DPD		5	0.28	0.68	-0.48	1.31	0.04
		Calcium_Pre	Calcium_Pre	5	1.20	1.10	0.00	2.10	1.90
		Calcium_Pre_Total		5	1.76	1.89	0.00	4.06	1.32
		Calcium_Post	Calcium_Post	5	1.26	1.18	0.00	2.50	1.80
		Calcium_Post_Total		5	1.33	1.42	0.00	2.93	1.01
		Calcium		5	-0.43	0.56	-1.36	0.00	-0.31
D	5	BAP_Post	BAP_Post	5	32.17	14.99	19.08	56.93	27.33
		BAP_Pre	BAP_Pre	5	30.50	12.29	21.68	51.14	23.85
		BAP		5	1.68	4.02	-4.77	5.80	2.03
		PTH_Post	PTH_Post	5	1.35	0.80	0.69	2.28	0.92
		PTH_Pre	PTH_Pre	5	1.25	0.47	0.61	1.65	1.51
		PTH		5	0.10	0.58	-0.68	0.77	0.08
		VitD_Post	VitD_Post	5	82.04	25.80	41.77	107.04	90.40
		VitD_Pre	VitD_Pre	5	67.43	22.90	35.48	99.08	66.37
		VitD		5	14.61	25.30	-26.30	32.89	28.33
		Osteocalcin_Post	Osteocalcin_Post	5	11.36	2.96	9.31	16.22	9.74
		Osteocalcin_Pre	Osteocalcin_Pre	5	10.82	1.98	9.19	13.91	10.19

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Osteocalcin		5	0.54	1.13	-0.82	2.31	0.52
DPD_Post	DPD_Post	5	7.84	0.93	6.82	9.08	7.88
DPD_Pre	DPD_Pre	5	7.87	1.63	5.43	9.68	8.05
DPD		5	-0.04	1.74	-2.62	1.75	-0.17
Calcium_Pre	Calcium_Pre	5	1.72	1.64	0.00	3.50	2.20
Calcium_Pre_Total		5	1.62	1.51	0.00	2.95	2.20
Calcium_Post	Calcium_Post	5	3.20	2.37	0.00	6.30	3.20
Calcium_Post_Total		5	3.12	2.38	0.00	6.23	3.06
Calcium		5	1.50	1.64	0.00	4.02	1.48

☆☆☆ƒ

Osteocalcin		5	0.54	1.13	-0.82	2.31	0.52
DPD_Post	DPD_Post	5	7.84	0.93	6.82	9.08	7.88
DPD_Pre	DPD_Pre	5	7.87	1.63	5.43	9.68	8.05

Table A- 6: Box plots of the change in the bone markers at the end of the trial

Boxplot of the change in BAP at the end of the trial according to treatment
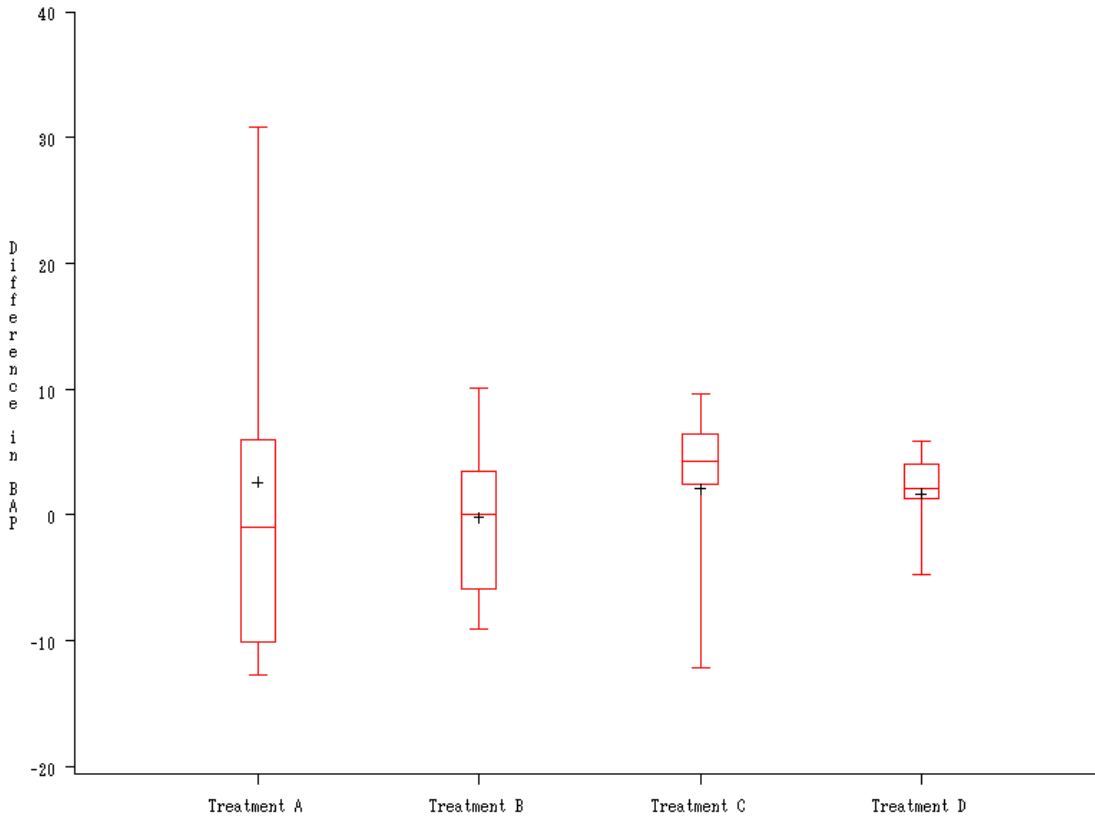
Boxplot of the change in PTH at the end of the trial according to treatment
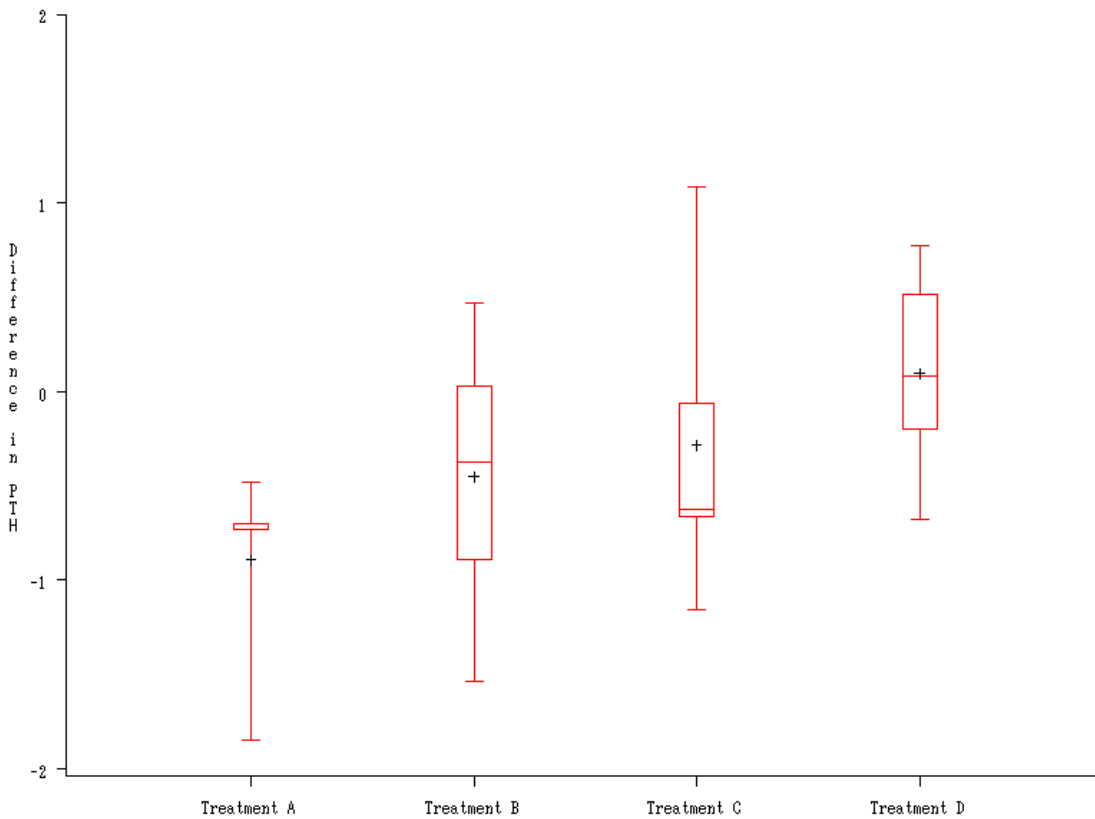
Boxplot of the change in VitD at the end of the trial according to treatment
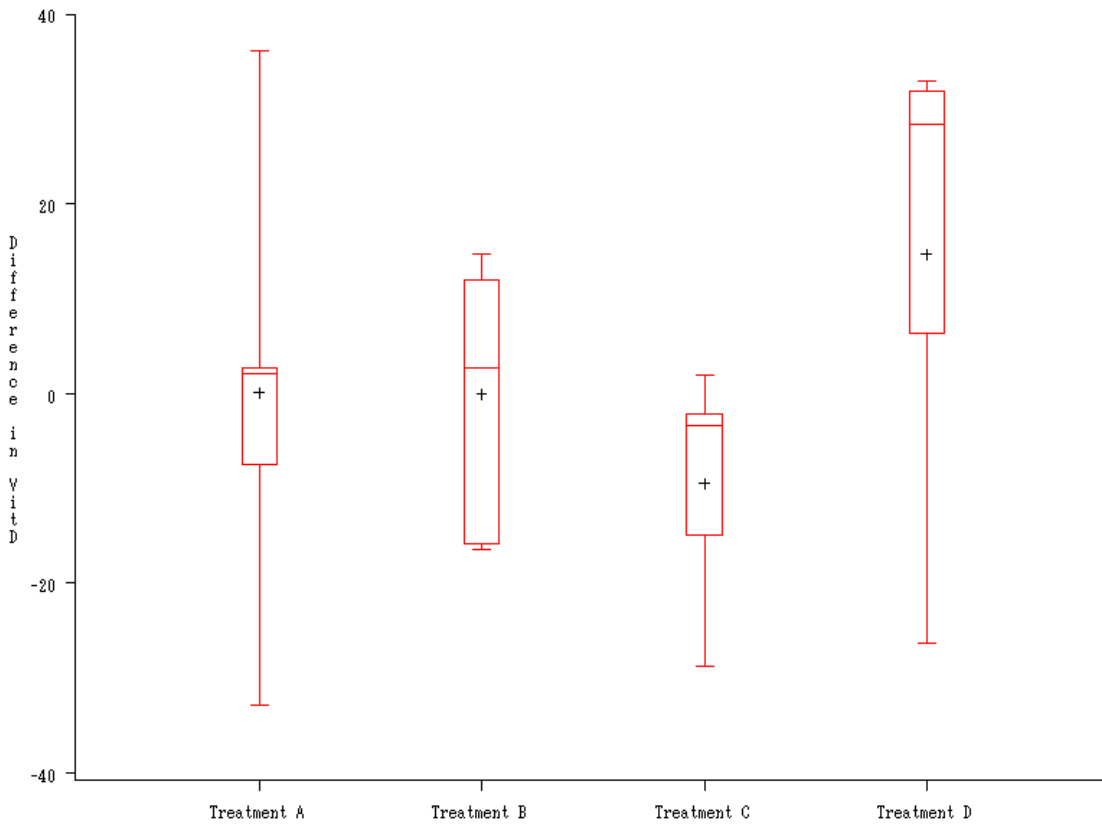
Boxplot of the change in Osteocalcin at the end of the trial according to treatment
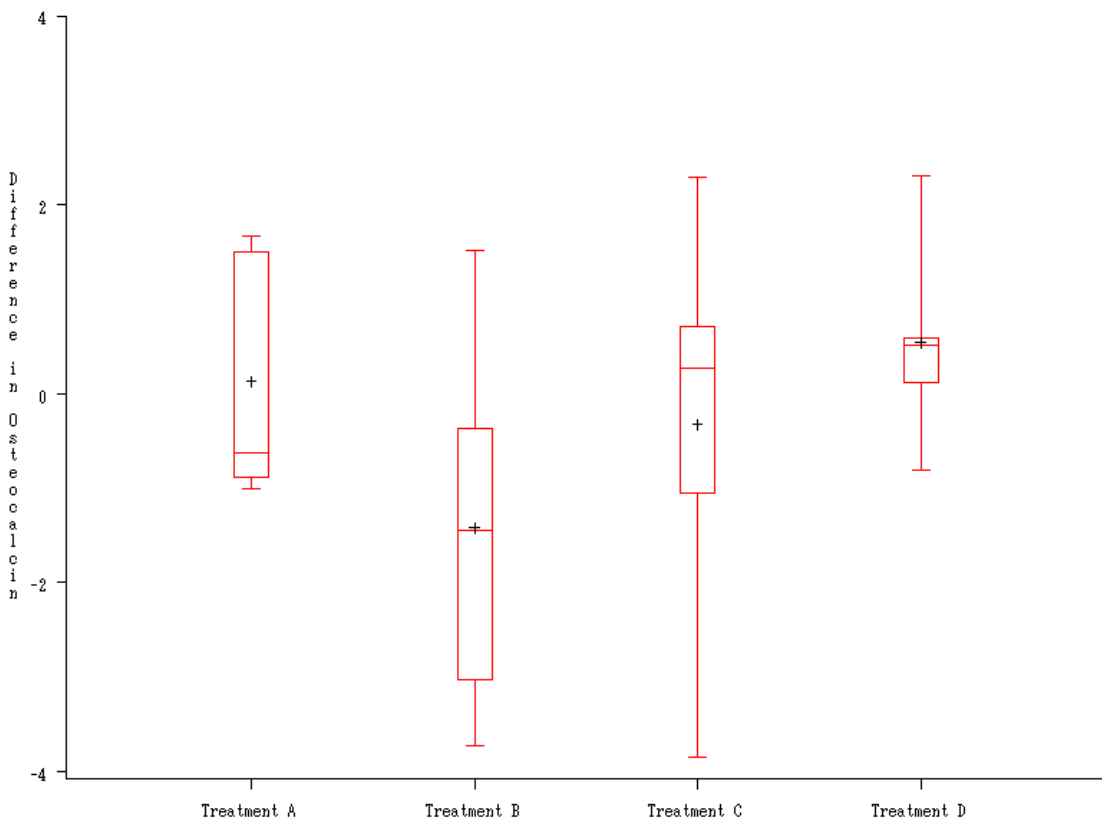
Boxplot of the change in DPD at the end of the trial according to treatment
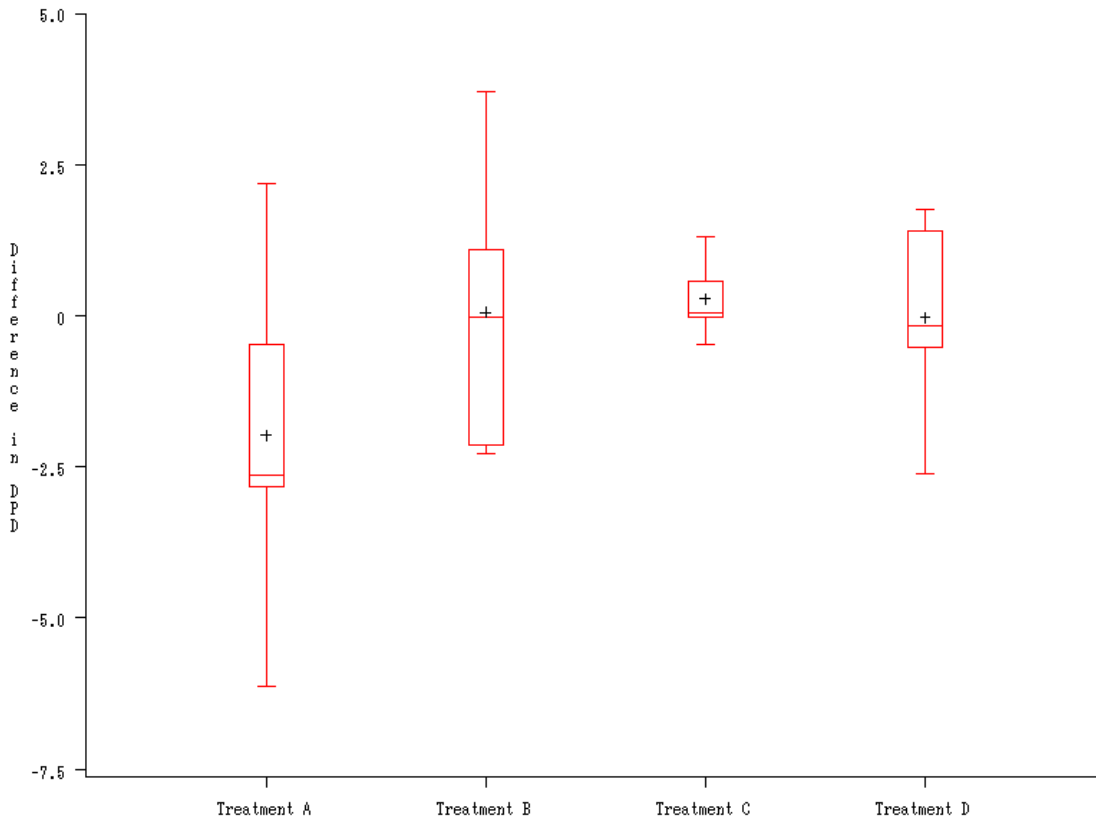
Boxplot of the change in Calcium at the end of the trial according to treatment
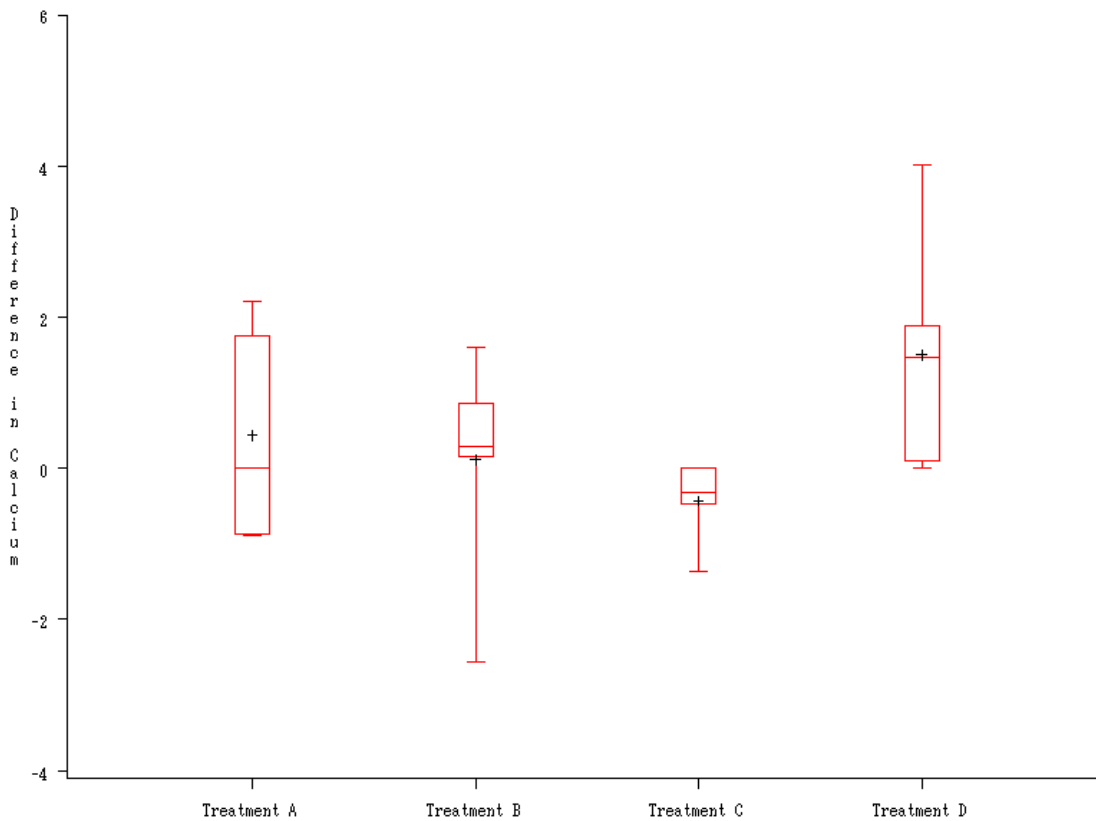
Table B- 1: SAS ANOVA listing for BAP

```
                      SC00204: Osteoporosis Study
                Treatment A= Aquamin F 600 mg (200 mg TID)
               Treatment B= Calcium Carbonate 600 mg (200 mg TID)
               Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                         Treatment D= Placebo
                      ANOVA of BAP using proc glm

                         Class Level Information

                 Class        Levels    Values
                 Treatment       4       A B C D

                 Number of Observations Read        21
                 Number of Observations Used        21
```

Dependent Variable: BAP

```
                                   Sum of
Source                   DF        Squares     Mean Square   F Value   Pr > F
Model                     3      25.794031       8.598010      0.08    0.9699
Error                    17    1821.351619     107.138331
Corrected Total          20    1847.145650

            R-Square      Coeff Var      Root MSE     BAP Mean
            0.013964      721.1880       10.35076     1.435238

Source                   DF       Type I SS     Mean Square   F Value   Pr > F
Treatment                 3     25.79403128      8.59801043      0.08    0.9699

Source                   DF      Type III SS    Mean Square   F Value   Pr > F
Treatment                 3     25.79403128      8.59801043      0.08    0.9699

                                            Standard
Parameter            Estimate               Error      t Value   Pr > |t|
Intercept          1.675600000 B          4.62900271     0.36     0.7218
Treatment A        0.901400000 B          6.54639841     0.14     0.8921
Treatment B       -1.921933333 B          6.26769930    -0.31     0.7628
Treatment C        0.395400000 B          6.54639841     0.06     0.9525
Treatment D        0.000000000 B              .            .        .
```

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

```
            Observation      Observed       Predicted       Residual
                 1         -1.04900000     2.57700000      -3.62600000
                 2         30.85400000     2.57700000      28.27700000
                 3          5.96500000     2.57700000       3.38800000
                 4        -10.10100000     2.57700000     -12.67800000
                 5        -12.78400000     2.57700000     -15.36100000
                 6         -5.92200000    -0.24633333      -5.67566667
                 7          3.19600000    -0.24633333       3.44233333
                 8          3.39400000    -0.24633333       3.64033333
                 9         10.11200000    -0.24633333      10.35833333
                10         -9.14700000    -0.24633333      -8.90066667
                11         -3.11100000    -0.24633333      -2.86466667
                12          4.21900000     2.07100000       2.14800000
                13          6.39000000     2.07100000       4.31900000
                14          2.39700000     2.07100000       0.32600000
                15          9.57100000     2.07100000       7.50000000
                16        -12.22200000     2.07100000     -14.29300000
                17          2.03000000     1.67560000       0.35440000
                18          1.27700000     1.67560000      -0.39860000
                19          5.79600000     1.67560000       4.12040000
                20         -4.77400000     1.67560000      -6.44960000
                21          4.04900000     1.67560000       2.37340000

            Sum of Residuals                              0.000000
            Sum of Squared Residuals                   1821.351619
            Sum of Squared Residuals - Error SS           0.000000
            First Order Autocorrelation                   0.025969
            Durbin-Watson D                               1.937751

            Level of            -------------BAP-------------
            Treatment     N          Mean            Std Dev
            A             5       2.57700000       17.4707409
            B             6      -0.24633333        7.0998328
            C             5       2.07100000        8.4248708
            D             5       1.67560000        4.0153625

                          Least Squares Means

                                 Standard                  LSMEAN
            Treatment  BAP LSMEAN    Error      Pr > |t|    Number
            A          2.57700000  4.62900271    0.5850       1
            B         -0.24633333  4.22568201    0.9542       2
            C          2.07100000  4.62900271    0.6602       3
            D          1.67560000  4.62900271    0.7218       4

            Least Squares Means for effect Treatment
              Pr > |t| for H0: LSMean(i)=LSMean(j)

                       Dependent Variable: BAP

        i/j         1         2         3         4
         1                 0.6581    0.9393    0.8921
         2       0.6581              0.7162    0.7628
         3       0.9393    0.7162              0.9525
         4       0.8921    0.7628    0.9525

            Treatment    BAP LSMEAN      90% Confidence Limits
            A             2.577000     -5.475644    10.629644
            B            -0.246333     -7.597358     7.104692
            C             2.071000     -5.981644    10.123644
            D             1.675600     -6.377044     9.728244

            Least Squares Means for Effect Treatment

                       Difference
                       Between       90% Confidence Limits for
            i     j      Means          LSMean(i)-LSMean(j)
            1     2     2.823333     -8.079999    13.726665
            1     3     0.506000    -10.882159    11.894159
            1     4     0.901400    -10.486759    12.289559
            2     3    -2.317333    -13.220665     8.585999
```

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

```
              2    4      -1.921933     -12.825265      8.981399
              3    4       0.395400     -10.992759     11.783559
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: BAP

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	21.73966667	21.73966667	0.20	0.6581
A vs C	1	0.64009000	0.64009000	0.01	0.9393
A vs D	1	2.03130490	2.03130490	0.02	0.8921

```
              2    4      -1.921933     -12.825265      8.981399
              3    4       0.395400     -10.992759     11.783559
```

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Table B- 2: SAS ANOVA listing for PTH

```
                SC00204: Osteoporosis Study
        Treatment A= Aquamin F 600 mg (200 mg TID)
       Treatment B= Calcium Carbonate 600 mg (200 mg TID)
     Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                  Treatment D= Placebo
              ANOVA of PTH using proc glm

                 Class Level Information

           Class        Levels     Values
           Treatment      4        A B C D

           Number of Observations Read       21
           Number of Observations Used       21
```

Dependent Variable: PTH

```
                               Sum of
Source               DF        Squares      Mean Square    F Value    Pr > F
Model                 3      2.53036842      0.84345614      1.78     0.1885
Error                17      8.04081253      0.47298897
Corrected Total      20     10.57118095

           R-Square     Coeff Var      Root MSE      PTH Mean
           0.239365    -177.7330       0.687742     -0.386952

Source               DF      Type I SS     Mean Square    F Value    Pr > F
Treatment             3     2.53036842      0.84345614      1.78     0.1885

Source               DF    Type III SS     Mean Square    F Value    Pr > F
Treatment             3     2.53036842      0.84345614      1.78     0.1885

                                Standard
Parameter          Estimate       Error      t Value    Pr > |t|
Intercept         0.0962000000 B  0.30756754    0.31      0.7583
Treatment A       -.9908000000 B  0.43496619   -2.28      0.0359
Treatment B       -.5465333333 B  0.41644842   -1.31      0.2068
Treatment C       -.3826000000 B  0.43496619   -0.88      0.3913
Treatment D        0.0000000000 B      .          .         .
```

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

```
Observation           Observed          Predicted          Residual
     1              -1.85400000        -0.89460000        -0.95940000
     2              -0.73000000        -0.89460000         0.16460000
     3              -0.70100000        -0.89460000         0.19360000
     4              -0.48500000        -0.89460000         0.40960000
     5              -0.70300000        -0.89460000         0.19160000
     6               0.02400000        -0.45033333         0.47433333
     7              -0.61500000        -0.45033333        -0.16466667
     8              -0.89600000        -0.45033333        -0.44566667
     9              -0.14300000        -0.45033333         0.30733333
    10              -1.54200000        -0.45033333        -1.09166667
    11               0.47000000        -0.45033333         0.92033333
    12              -0.62900000        -0.28640000        -0.34260000
    13              -0.66500000        -0.28640000        -0.37860000
    14              -1.15700000        -0.28640000        -0.87060000
    15               1.08200000        -0.28640000         1.36840000
    16              -0.06300000        -0.28640000         0.22340000
    17              -0.20300000         0.09620000        -0.29920000
    18               0.77300000         0.09620000         0.67680000
    19              -0.68200000         0.09620000        -0.77820000
    20               0.51200000         0.09620000         0.41580000
    21               0.08100000         0.09620000        -0.01520000

           Sum of Residuals                           0.00000000
           Sum of Squared Residuals                   8.04081253
           Sum of Squared Residuals - Error SS       -0.00000000
           First Order Autocorrelation               -0.40130527
           Durbin-Watson D                            2.68810974

      Level of           -------------PTH-------------
      Treatment     N         Mean              Std Dev
      A             5      -0.89460000        0.54532220
      B             6      -0.45033333        0.71887820
      C             5      -0.28640000        0.85747933
      D             5       0.09620000        0.57582437

                      Least Squares Means

                                 Standard                    LSMEAN
Treatment     PTH LSMEAN          Error      Pr > |t|        Number
A            -0.89460000        0.30756754    0.0098            1
B            -0.45033333        0.28076947    0.1271            2
C            -0.28640000        0.30756754    0.3648            3
D             0.09620000        0.30756754    0.7583            4

           Least Squares Means for effect Treatment
             Pr > |t| for H0: LSMean(i)=LSMean(j)

                  Dependent Variable: PTH

   i/j          1          2          3          4
    1                    0.3010     0.1800     0.0359
    2        0.3010                 0.6987     0.2068
    3        0.1800     0.6987                 0.3913
    4        0.0359     0.2068     0.3913

      Treatment     PTH LSMEAN      90% Confidence Limits
      A            -0.894600      -1.429647     -0.359553
      B            -0.450333      -0.938762      0.038095
      C            -0.286400      -0.821447      0.248647
      D             0.096200      -0.438847      0.631247

           Least Squares Means for Effect Treatment

                       Difference
                        Between      90% Confidence Limits for
      i     j            Means          LSMean(i)-LSMean(j)
      1     2          -0.444267      -1.168723      0.280190
      1     3          -0.608200      -1.364870      0.148470
      1     4          -0.990800      -1.747470     -0.234130
```

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

```
          2    3      -0.163933      -0.888390      0.560523
          2    4      -0.546533      -1.270990      0.177923
          3    4      -0.382600      -1.139270      0.374070
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: PTH

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	0.53828965	0.53828965	1.14	0.3010
A vs C	1	0.92476810	0.92476810	1.96	0.1800
A vs D	1	2.45421160	2.45421160	5.19	0.0359
B vs C	1	0.07329310	0.07329310	0.15	0.6987
B vs D	1	0.81463278	0.81463278	1.72	0.2068
C vs D	1	0.36595690	0.36595690	0.77	0.3913

```
          2    3      -0.163933      -0.888390      0.560523
          2    4      -0.546533      -1.270990      0.177923
          3    4      -0.382600      -1.139270      0.374070
```

A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium supplementation on markers of bone turnover

Table B- 3: SAS ANOVA listing for Vitamin D

```
                       SC00204: Osteoporosis Study
                   Treatment A= Aquamin F 600 mg (200 mg TID)
                 Treatment B= Calcium Carbonate 600 mg (200 mg TID)
               Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                         Treatment D= Placebo
                       ANOVA of VitD using proc glm

                         Class Level Information

                 Class           Levels    Values
                 Treatment          4      A B C D

                 Number of Observations Read         21
                 Number of Observations Used         21
```

Dependent Variable: VitD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1484.039160	494.679720	1.26	0.3198
Error	17	6678.895350	392.876197		
Corrected Total	20	8162.934511			

R-Square	Coeff Var	Root MSE	VitD Mean
0.181802	1613.658	19.82110	1.228333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	1484.039160	494.679720	1.26	0.3198

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	1484.039160	494.679720	1.26	0.3198

Parameter	Estimate		Standard Error	t Value	Pr > |t|
Intercept	14.60760000	B	8.86426756	1.65	0.1177
Treatment A	-14.51060000	B	12.53596741	-1.16	0.2631
Treatment B	-14.66760000	B	12.00227502	-1.22	0.2384
Treatment C	-24.08120000	B	12.53596741	-1.92	0.0717
Treatment D	0.00000000	B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Observation	Observed	Predicted	Residual
1	-7.49700000	0.09700000	-7.59400000
2	2.04400000	0.09700000	1.94700000
3	36.09200000	0.09700000	35.99500000
4	2.69300000	0.09700000	2.59600000
5	-32.84700000	0.09700000	-32.94400000
6	-15.90400000	-0.06000000	-15.84400000
7	11.89500000	-0.06000000	11.95500000
8	11.30000000	-0.06000000	11.36000000
9	14.72400000	-0.06000000	14.78400000
10	-16.52500000	-0.06000000	-16.46500000
11	-5.85000000	-0.06000000	-5.79000000
12	1.88100000	-9.47360000	11.35460000
13	-28.79200000	-9.47360000	-19.31840000
14	-14.92100000	-9.47360000	-5.44740000
15	-2.18700000	-9.47360000	7.28660000
16	-3.34900000	-9.47360000	6.12460000
17	32.89300000	14.60760000	18.28540000
18	28.32800000	14.60760000	13.72040000
19	31.81700000	14.60760000	17.20940000
20	6.29900000	14.60760000	-8.30860000
21	-26.29900000	14.60760000	-40.90660000

```
             Sum of Residuals                       0.000000
             Sum of Squared Residuals            6678.895350
             Sum of Squared Residuals - Error SS     0.000000
             First Order Autocorrelation            0.175537
             Durbin-Watson D                        1.389749
```

Level of Treatment	N	Mean	Std Dev
A	5	0.0970000	24.7444237
B	6	-0.0600000	14.4650679
C	5	-9.4736000	12.4740426
D	5	14.6076000	25.3039100

Least Squares Means

Treatment	VitD LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.0970000	8.8642676	0.9914	1
B	-0.0600000	8.0919322	0.9942	2
C	-9.4736000	8.8642676	0.3001	3
D	14.6076000	8.8642676	0.1177	4

Least Squares Means for effect Treatment
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: VitD

i/j	1	2	3	4
1		0.9897	0.4557	0.2631
2	0.9897		0.4436	0.2384
3	0.4557	0.4436		0.0717
4	0.2631	0.2384	0.0717	

Treatment	VitD LSMEAN	90% Confidence Limits	
A	0.097000	-15.323339	15.517339
B	-0.060000	-14.136780	14.016780
C	-9.473600	-24.893939	5.946739
D	14.607600	-0.812739	30.027939

Least Squares Means for Effect Treatment

i	j	Difference Between Means	90% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	0.157000	-20.722238	21.036238
1	3	9.570600	-12.237053	31.378253
1	4	-14.510600	-36.318253	7.297053

```
              2    3      9.413600     -11.465638    30.292838
              2    4    -14.667600     -35.546838     6.211638
              3    4    -24.081200     -45.888853    -2.273547
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: VitD

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	0.0672245	0.0672245	0.00	0.9897
A vs C	1	228.9909609	228.9909609	0.58	0.4557
A vs D	1	526.3937809	526.3937809	1.34	0.2631

```
              2    3      9.413600     -11.465638    30.292838
              2    4    -14.667600     -35.546838     6.211638
              3    4    -24.081200     -45.888853    -2.273547
```

Table B- 4: SAS ANOVA listing for Osteocalin

```
                    SC00204: Osteoporosis Study
                Treatment A= Aquamin F 600 mg (200 mg TID)
                Treatment B= Calcium Carbonate 600 mg (200 mg TID)
                Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                        Treatment D= Placebo
                  ANOVA of Osteocalcin using proc glm

                        Class Level Information

                Class         Levels    Values
                Treatment        4       A B C D

                Number of Observations Read        21
                Number of Observations Used        21
```

Dependent Variable: Osteocalcin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	11.98505910	3.99501970	1.29	0.3098
Error	17	52.63578613	3.09622271		
Corrected Total	20	64.62084524			

R-Square	Coeff Var	Root MSE	Osteocalcin Mean
0.185467	-543.4086	1.759609	-0.323810

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	11.98505910	3.99501970	1.29	0.3098

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	11.98505910	3.99501970	1.29	0.3098

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	0.542800000 B	0.78692093	0.69	0.4996
Treatment A	-0.413000000 B	1.11287425	-0.37	0.7151
Treatment B	-1.962133333 B	1.06549597	-1.84	0.0831
Treatment C	-0.872200000 B	1.11287425	-0.78	0.4440
Treatment D	0.000000000 B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Observation	Observed	Predicted	Residual
1	1.66600000	0.12980000	1.53620000
2	-1.00900000	0.12980000	-1.13880000
3	-0.88300000	0.12980000	-1.01280000
4	-0.62800000	0.12980000	-0.75780000
5	1.50300000	0.12980000	1.37320000
6	-3.03600000	-1.41933333	-1.61666667
7	-2.26200000	-1.41933333	-0.84266667
8	1.52000000	-1.41933333	2.93933333
9	-0.36500000	-1.41933333	1.05433333
10	-3.73500000	-1.41933333	-2.31566667
11	-0.63800000	-1.41933333	0.78133333
12	0.70600000	-0.32940000	1.03540000
13	0.26900000	-0.32940000	0.59840000
14	-3.86000000	-0.32940000	-3.53060000
15	2.29300000	-0.32940000	2.62240000
16	-1.05500000	-0.32940000	-0.72560000
17	0.59100000	0.54280000	0.04820000
18	0.51800000	0.54280000	-0.02480000
19	2.30600000	0.54280000	1.76320000
20	-0.81900000	0.54280000	-1.36180000
21	0.11800000	0.54280000	-0.42480000

```
            Sum of Residuals                      0.00000000
            Sum of Squared Residuals             52.63578613
            Sum of Squared Residuals - Error SS   0.00000000
            First Order Autocorrelation          -0.36293961
            Durbin-Watson D                       2.67761613
```

Level of Treatment	N	Osteocalcin Mean	Std Dev
A	5	0.12980000	1.33627082
B	6	-1.41933333	1.95180897
C	5	-0.32940000	2.30752840
D	5	0.54280000	1.13432434

Least Squares Means

Treatment	Osteocalcin LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.12980000	0.78692093	0.8709	1
B	-1.41933333	0.71835724	0.0646	2
C	-0.32940000	0.78692093	0.6808	3
D	0.54280000	0.78692093	0.4996	4

Least Squares Means for effect Treatment
Pr > |t| for HO: LSMean(i)=LSMean(j)

Dependent Variable: Osteocalcin

i/j	1	2	3	4
1		0.1642	0.6850	0.7151
2	0.1642		0.3207	0.0831
3	0.6850	0.3207		0.4440
4	0.7151	0.0831	0.4440	

Treatment	Osteocalcin LSMEAN	90% Confidence Limits	
A	0.129800	-1.239133	1.498733
B	-1.419333	-2.668992	-0.169674
C	-0.329400	-1.698333	1.039533
D	0.542800	-0.826133	1.911733

Least Squares Means for Effect Treatment

i	j	Difference Between Means	90% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	1.549133	-0.304411	3.402677
1	3	0.459200	-1.476764	2.395164

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```
                      1    4    -0.413000    -2.348964     1.522964
                      2    3    -1.089933    -2.943477     0.763611
                      2    4    -1.962133    -3.815677    -0.108589
                      3    4    -0.872200    -2.808164     1.063764
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: Osteocalcin

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	6.54494750	6.54494750	2.11	0.1642
A vs C	1	0.52716160	0.52716160	0.17	0.6850
A vs D	1	0.42642250	0.42642250	0.14	0.7151

```
                      1    4    -0.413000    -2.348964     1.522964
                      2    3    -1.089933    -2.943477     0.763611
                      2    4    -1.962133    -3.815677    -0.108589
```

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Table B- 5: SAS ANOVA listing for DPD

```
              SCO0204: Osteoporosis Study
         Treatment A= Aquamin F 600 mg (200 mg TID)
         Treatment B= Calcium Carbonate 600 mg (200 mg TID)
       Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                   Treatment D= Placebo
              ANOVA of DPD using proc glm

                Class Level Information

         Class          Levels    Values
         Treatment        4       A B C D

         Number of Observations Read        21
         Number of Observations Used        21
```

Dependent Variable: DPD

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	16.66240429	5.55413476	1.20	0.3411
Error	17	78.94131000	4.64360647		
Corrected Total	20	95.60371429			

R-Square	Coeff Var	Root MSE	DPD Mean
0.174286	-544.5603	2.154903	-0.395714

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	16.66240429	5.55413476	1.20	0.3411

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	16.66240429	5.55413476	1.20	0.3411

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	-0.036000000 B	0.96370187	-0.04	0.9706
Treatment A	-1.940000000 B	1.36288025	-1.42	0.1727
Treatment B	0.091000000 B	1.30485850	0.07	0.9452
Treatment C	0.320000000 B	1.36288025	0.23	0.8172
Treatment D	0.000000000 B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Observation	Observed	Predicted	Residual
1	2.18000000	-1.97600000	4.15600000
2	-2.83000000	-1.97600000	-0.85400000
3	-6.12000000	-1.97600000	-4.14400000
4	-0.47000000	-1.97600000	1.50600000
5	-2.64000000	-1.97600000	-0.66400000
6	-2.28000000	0.05500000	-2.33500000
7	1.10000000	0.05500000	1.04500000
8	3.71000000	0.05500000	3.65500000
9	-1.10000000	0.05500000	-1.15500000
10	-2.14000000	0.05500000	-2.19500000
11	1.04000000	0.05500000	0.98500000
12	0.04000000	0.28400000	-0.24400000
13	1.31000000	0.28400000	1.02600000
14	0.57000000	0.28400000	0.28600000
15	-0.02000000	0.28400000	-0.30400000
16	-0.48000000	0.28400000	-0.76400000
17	1.75000000	-0.03600000	1.78600000
18	-2.62000000	-0.03600000	-2.58400000
19	-0.53000000	-0.03600000	-0.49400000
20	-0.17000000	-0.03600000	-0.13400000
21	1.39000000	-0.03600000	1.42600000

```
Sum of Residuals                        -0.00000000
Sum of Squared Residuals                78.94131000
Sum of Squared Residuals - Error SS     -0.00000000
First Order Autocorrelation             -0.16530619
Durbin-Watson D                          2.08605332
```

Level of Treatment	N	DPD Mean	DPD Std Dev
A	5	-1.97600000	3.07747136
B	6	0.05500000	2.32488924
C	5	0.28400000	0.68376165
D	5	-0.03600000	1.74372590

Least Squares Means

Treatment	DPD LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	-1.97600000	0.96370187	0.0561	1
B	0.05500000	0.87973542	0.9509	2
C	0.28400000	0.96370187	0.7718	3
D	-0.03600000	0.96370187	0.9706	4

Least Squares Means for effect Treatment
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: DPD

i/j	1	2	3	4
1		0.1380	0.1156	0.1727
2	0.1380		0.8628	0.9452
3	0.1156	0.8628		0.8172
4	0.1727	0.9452	0.8172	

Treatment	DPD LSMEAN	90% Confidence Limits	
A	-1.976000	-3.652462	-0.299538
B	0.055000	-1.475394	1.585394
C	0.284000	-1.392462	1.960462
D	-0.036000	-1.712462	1.640462

Least Squares Means for Effect Treatment

i	j	Difference Between Means	90% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	-2.031000	-4.300941	0.238941
1	3	-2.260000	-4.630876	0.110876
1	4	-1.940000	-4.310876	0.430876

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2	3	-0.229000	-2.498941	2.040941
2	4	0.091000	-2.178941	2.360941
3	4	0.320000	-2.050876	2.690876

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: DPD

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	11.24989364	11.24989364	2.42	0.1380
A vs C	1	12.76900000	12.76900000	2.75	0.1156
A vs D	1	9.40900000	9.40900000	2.03	0.1727

2	3	-0.229000	-2.498941	2.040941
2	4	0.091000	-2.178941	2.360941
3	4	0.320000	-2.050876	2.690876

Table B- 6: SAS ANOVA listing for Calcium

```
                      SC00204: Osteoporosis Study
                   Treatment A= Aquamin F 600 mg (200 mg TID)
                 Treatment B= Calcium Carbonate 600 mg (200 mg TID)
               Treatment C= Tricalcium Phosphate 600 mg (200 mg TID)
                          Treatment D= Placebo
                      ANOVA of Calcium using proc glm

                          Class Level Information

                   Class          Levels    Values
                   Treatment         4       A B C D

                    Number of Observations Read          21
                    Number of Observations Used          21
```

Dependent Variable: Calcium

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	9.98682371	3.32894124	1.85	0.1760
Error	17	30.55136293	1.79713900		
Corrected Total	20	40.53818664			

R-Square	Coeff Var	Root MSE	Calcium Mean
0.246356	343.2340	1.340574	0.390571

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Treatment	3	9.98682371	3.32894124	1.85	0.1760

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	3	9.98682371	3.32894124	1.85	0.1760

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Intercept	1.499100000 B	0.59952298	2.50	0.0229
Treatment A	-1.060500000 B	0.84785352	-1.25	0.2279
Treatment B	-1.389433333 B	0.81175795	-1.71	0.1051
Treatment C	-1.928000000 B	0.84785352	-2.27	0.0362
Treatment D	0.000000000 B	.	.	.

NOTE: The X'X matrix has been found to be singular, and a generalized inverse was used to solve the normal equations. Terms whose estimates are followed by the letter 'B' are not uniquely estimable.

Observation	Observed	Predicted	Residual
1	-0.88000000	0.43860000	-1.31860000
2	-0.89800000	0.43860000	-1.33660000
3	2.21000000	0.43860000	1.77140000
4	0.00000000	0.43860000	-0.43860000
5	1.76100000	0.43860000	1.32240000
6	0.85100000	0.10966667	0.74133333
7	1.60800000	0.10966667	1.49833333
8	0.16000000	0.10966667	0.05033333
9	-2.55600000	0.10966667	-2.66566667
10	0.18000000	0.10966667	0.07033333
11	0.41500000	0.10966667	0.30533333
12	-0.47500000	-0.42890000	-0.04610000
13	-0.31300000	-0.42890000	0.11590000
14	-1.35650000	-0.42890000	-0.92760000
15	0.00000000	-0.42890000	0.42890000
16	0.00000000	-0.42890000	0.42890000
17	4.02200000	1.49910000	2.52290000
18	1.89000000	1.49910000	0.39090000
19	0.10750000	1.49910000	-1.39160000
20	0.00000000	1.49910000	-1.49910000
21	1.47600000	1.49910000	-0.02310000

```
            Sum of Residuals                      0.00000000
            Sum of Squared Residuals             30.55136293
            Sum of Squared Residuals - Error SS  -0.00000000
            First Order Autocorrelation           0.10501784
            Durbin-Watson D                        1.73303594
```

Level of Treatment	N	Calcium Mean	Std Dev
A	5	0.43860000	1.46664372
B	6	0.10966667	1.41428889
C	5	-0.42890000	0.55765361
D	5	1.49910000	1.63571179

Least Squares Means

Treatment	Calcium LSMEAN	Standard Error	Pr > |t|	LSMEAN Number
A	0.43860000	0.59952298	0.4744	1
B	0.10966667	0.54728710	0.8436	2
C	-0.42890000	0.59952298	0.4841	3
D	1.49910000	0.59952298	0.0229	4

```
                Least Squares Means for effect Treatment
                 Pr > |t| for H0: LSMean(i)=LSMean(j)

                      Dependent Variable: Calcium
```

i/j	1	2	3	4
1		0.6904	0.3206	0.2279
2	0.6904		0.5159	0.1051
3	0.3206	0.5159		0.0362
4	0.2279	0.1051	0.0362	

Treatment	Calcium LSMEAN	90% Confidence Limits	
A	0.438600	-0.604334	1.481534
B	0.109667	-0.842398	1.061731
C	-0.428900	-1.471834	0.614034
D	1.499100	0.456166	2.542034

Least Squares Means for Effect Treatment

i	j	Difference Between Means	90% Confidence Limits for LSMean(i)-LSMean(j)	
1	2	0.328933	-1.083206	1.741073
1	3	0.867500	-0.607432	2.342432

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```
        1    4      -1.060500      -2.535432       0.414432
        2    3       0.538567      -0.873573       1.950706
        2    4      -1.389433      -2.801573       0.022706
        3    4      -1.928000      -3.402932      -0.453068
```

NOTE: To ensure overall protection level, only probabilities associated with pre-planned comparisons should be used.

Dependent Variable: Calcium

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
A vs B	1	0.29508310	0.29508310	0.16	0.6904
A vs C	1	1.88139063	1.88139063	1.05	0.3206
A vs D	1	2.81165062	2.81165062	1.56	0.2279

```
        1    4      -1.060500      -2.535432       0.414432
        2    3       0.538567      -0.873573       1.950706
        2    4      -1.389433      -2.801573       0.022706
        3    4      -1.928000      -3.402932      -0.453068
```

Table C- 1: Osteoporosis data set, residuals and studentised residuals

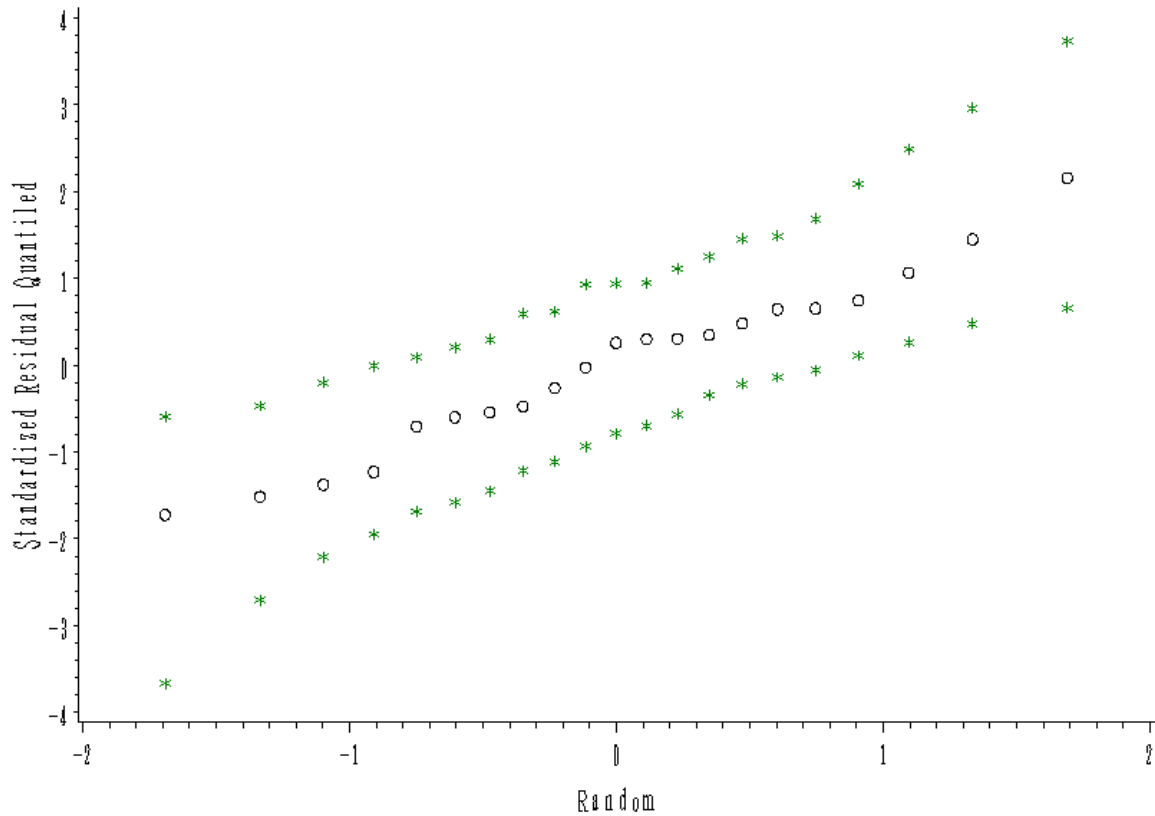| Vol. No.	BAP			PTH			Vitamin D		
	Data	Res.	Stud Res.	Data	Res.	Stud Res.	Data	Res.	Stud Res.
1	2.03	0.35	0.04	-0.20	-0.30	-0.49	32.89	18.29	1.03
2	1.28	-0.40	-0.04	0.77	0.68	1.10	28.33	13.72	0.77
3	-5.92	-5.68	-0.60	0.02	0.47	0.76	-15.90	-15.84	-0.88
4	-1.05	-3.63	-0.39	-1.85	-0.96	-1.56	-7.50	-7.59	-0.43
5	4.22	2.15	0.23	-0.63	-0.34	-0.56	1.88	11.35	0.64
6	30.85	28.28	3.05	-0.73	0.16	0.27	2.04	1.95	0.11
7	5.80	4.12	0.45	-0.68	-0.78	-1.27	31.82	17.21	0.97
8	6.39	4.32	0.47	-0.67	-0.38	-0.62	-28.79	-19.32	-1.09
9	3.20	3.44	0.36	-0.62	-0.16	-0.26	11.90	11.96	0.66
10	2.40	0.33	0.04	-1.16	-0.87	-1.42	-14.92	-5.45	-0.31
11	5.97	3.39	0.37	-0.70	0.19	0.31	36.09	36.00	2.03
12	3.39	3.64	0.39	-0.90	-0.45	-0.71	11.30	11.36	0.63
13	9.57	7.50	0.81	1.08	1.37	2.22	-2.19	7.29	0.41
14	10.11	10.36	1.10	-0.14	0.31	0.49	14.72	14.78	0.82
15	-12.22	-14.29	-1.54	-0.06	0.22	0.36	-3.35	6.12	0.35
16	-4.77	-6.45	-0.70	0.51	0.42	0.68	6.30	-8.31	-0.47
17	-10.10	-12.68	-1.37	-0.49	0.41	0.67	2.69	2.60	0.15
18	-9.15	-8.90	-0.94	-1.54	-1.09	-1.74	-16.53	-16.47	-0.91
19	-12.78	-15.36	-1.66	-0.70	0.19	0.31	-32.85	-32.94	-1.86
20	-3.11	-2.86	-0.30	0.47	0.92	1.47	-5.85	-5.79	-0.32
21	4.05	2.37	0.26	0.08	-0.02	-0.02	-26.30	-40.91	-2.31

Vol. No.	Osteocalcin			DPD			Calcium		
	Data	Res.	Stud Res.	Data	Res.	Stud Res.	Data	Res.	Stud Res.
1	0.59	0.05	0.03	1.75	1.79	0.93	4.02	2.52	2.10
2	0.52	-0.02	-0.02	-2.62	-2.58	-1.34	1.89	0.39	0.33
3	-3.04	-1.62	-1.01	-2.28	-2.34	-1.19	0.85	0.74	0.61
4	1.67	1.54	0.98	2.18	4.16	2.16	-0.88	-1.32	-1.10
5	0.71	1.04	0.66	0.04	-0.24	-0.13	-0.48	-0.05	-0.04
6	-1.01	-1.14	-0.72	-2.83	-0.85	-0.44	-0.90	-1.34	-1.11
7	2.31	1.76	1.12	-0.53	-0.49	-0.26	0.11	-1.39	-1.16
8	0.27	0.60	0.38	1.31	1.03	0.53	-0.31	0.12	0.10
9	-2.26	-0.84	-0.52	1.10	1.05	0.53	1.61	1.50	1.22
10	-3.86	-3.53	-2.24	0.57	0.29	0.15	-1.36	-0.93	-0.77
11	-0.88	-1.01	-0.64	-6.12	-4.14	-2.15	2.21	1.77	1.48
12	1.52	2.94	1.83	3.71	3.66	1.86	0.16	0.05	0.04
13	2.29	2.62	1.67	-0.02	-0.30	-0.16	0.00	0.43	0.36
14	-0.37	1.05	0.66	-1.10	-1.16	-0.59	-2.56	-2.67	-2.18
15	-1.06	-0.73	-0.46	-0.48	-0.76	-0.40	0.00	0.43	0.36
16	-0.82	-1.36	-0.87	-0.17	-0.13	-0.07	0.00	-1.50	-1.25
17	-0.63	-0.76	-0.48	-0.47	1.51	0.78	0.00	-0.44	-0.37
18	-3.74	-2.32	-1.44	-2.14	-2.20	-1.12	0.18	0.07	0.06
19	1.50	1.37	0.87	-2.64	-0.66	-0.34	1.76	1.32	1.10
20	-0.64	0.78	0.49	1.04	0.99	0.50	0.42	0.31	0.25
21	0.12	-0.42	-0.27	1.39	1.43	0.74	1.48	-0.02	-0.02

Study No. SC00204. Report.
A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium
supplementation on markers of bone turnover

Table D- 1: QQ-plots to assess the assumptions underlying the ANOVA model

Normal Quantile Plot for BAP

Normal Quantile Plot for PTH

Study No. SC00204. Report.
A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium
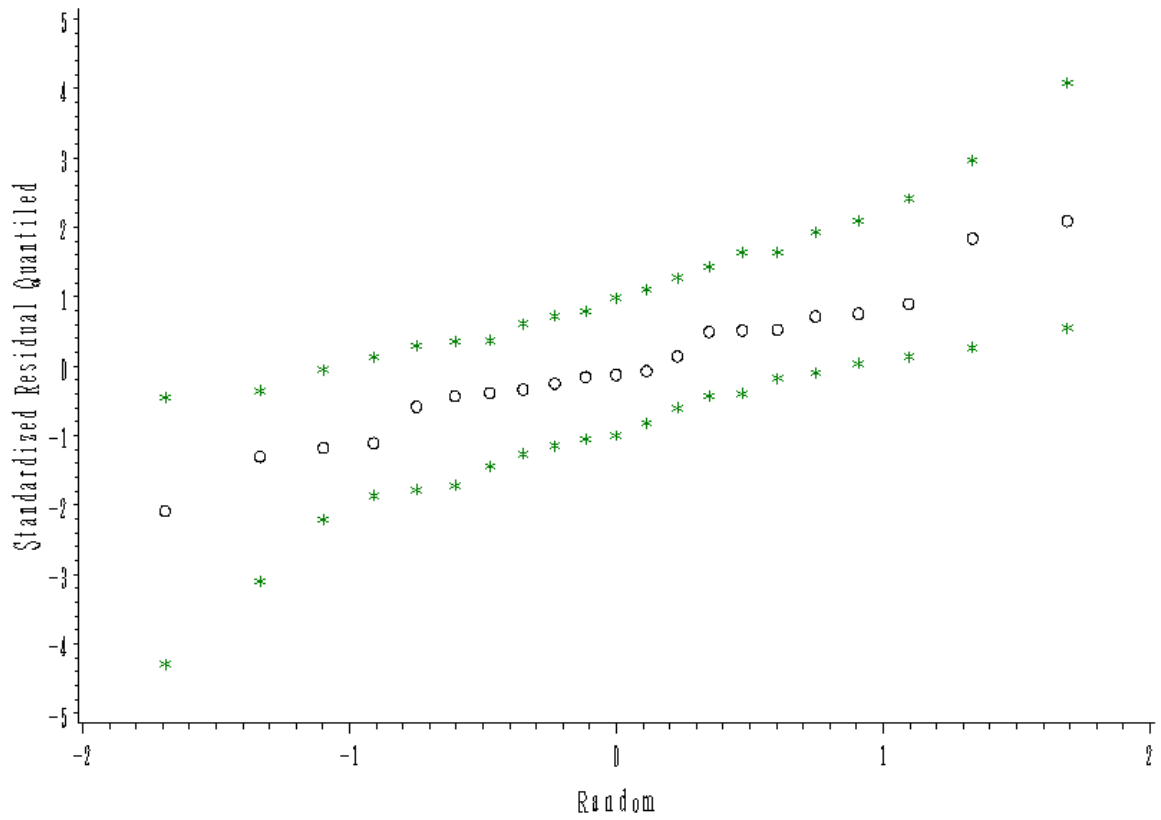supplementation on markers of bone turnover
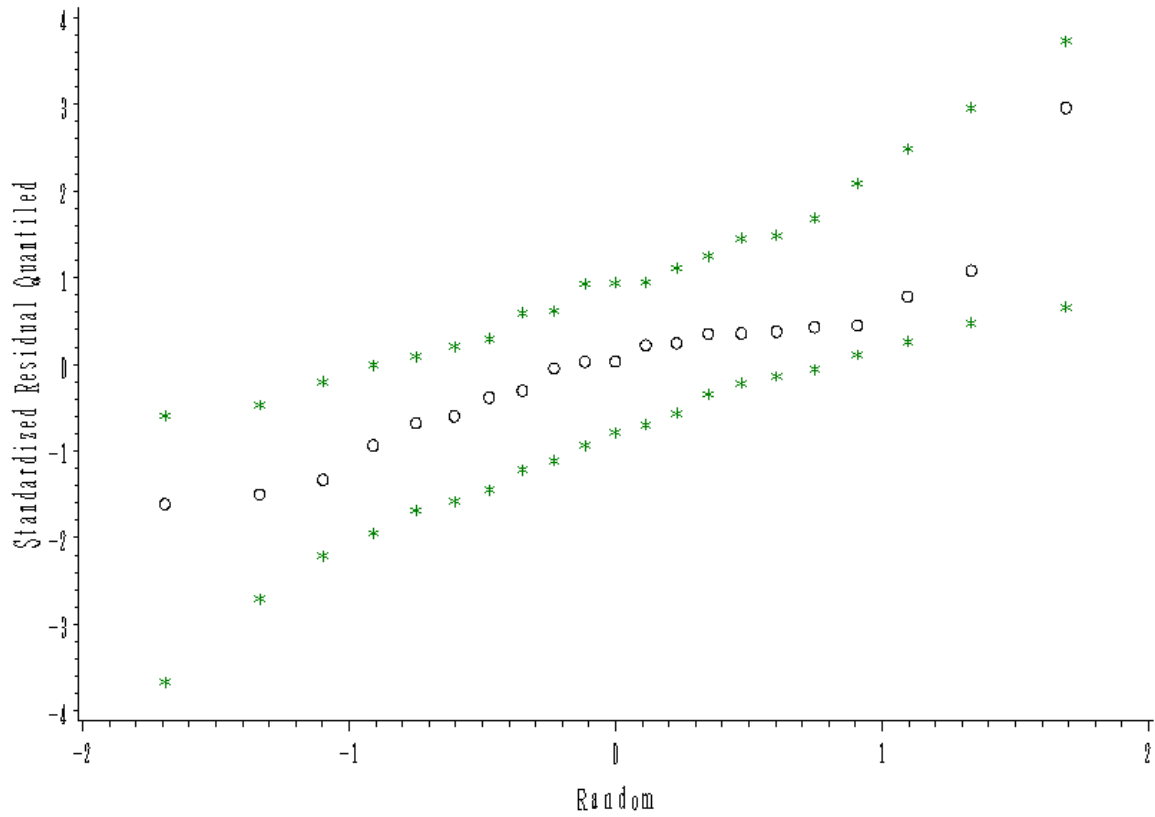
Normal Quantile Plot for VitD
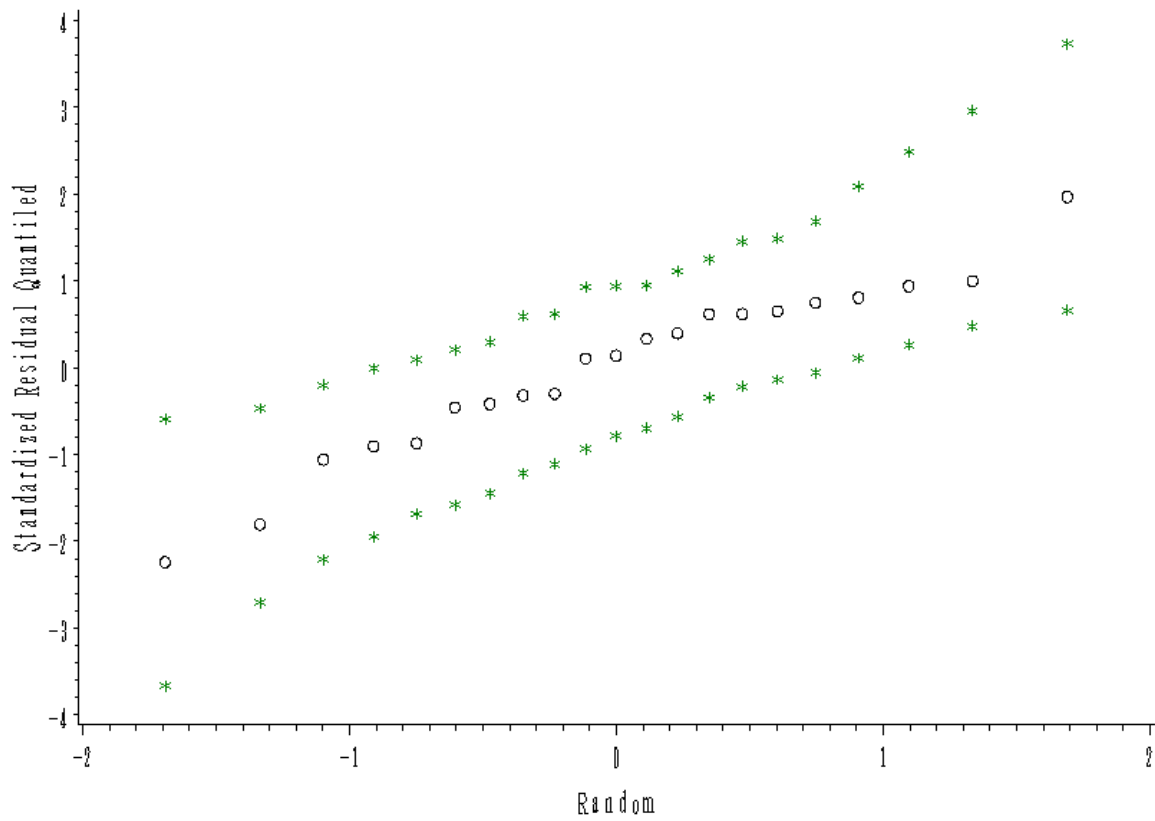
Normal Quantile Plot for Osteocalcin

Study No. SC00204. Report.
A double-blind placebo-controlled parallel group study in postmenopausal women to assess the effect of calcium
supplementation on markers of bone turnover
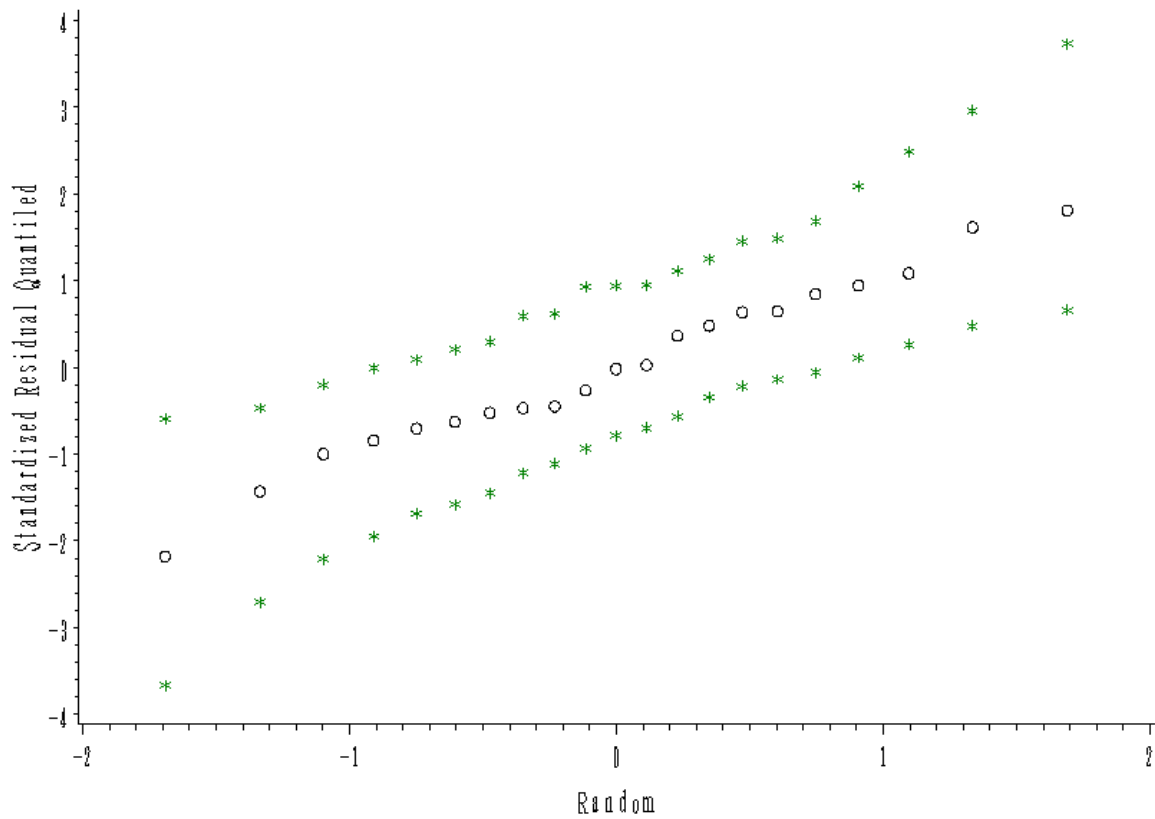
Normal Quantile Plot for DPD

Normal Quantile Plot for Calcium