IMPORTANT NOTE FROM THE EARTHING INSTITUTE:

The animal study presented below was designed to compare the lifetime effects of grounding vs. non-grounding on caged female rats in a research laboratory. However, critical differences in the cage environment used for grounded and non-grounded rats discouraged the study from being submitted to peer-reviewed journals for publication.

The flaws are as follows:

1) The standard shoebox cages used to house grounded animals were equipped with stainless steel mesh flooring electrically grounded to the building ground. There was no such flooring used in the cages of non-grounded rats.
2) The grounded cages bedding was minimized so that at least one paw was always touching the grounded mesh flooring. The animals in ungrounded cages had normal bedding.

After analyzing these results carefully, we came to the conclusion that these critical differences resulted in more stress for grounded animals as well as hind paw lesions that were observed on 10 of the 30 grounded animals.

The researcher wrote in section 5.3. *Interpretation* that it was "unlikely that these lesions would have affected any of the parameters measured in this study due to their localized nature." However, there is no way to know for sure with the present study design. It is reasonable to infer that this situation may have resulted in inflammation markers increase in all the grounded animals especially after the first 169 days as the animals became older. Results were analyzed after the first 6 months of the study and a second analysis was performed at the end of the study. Results for both are presented in the present report. The proper protocol should have required the ungrounded group cages to have an identical stainless steel mesh flooring as the grounded animals (but not electrically grounded) and also to have similar bedding.

The study still provides intriguing results and, with a corrected design, could be used as a guideline for future studies.

FINAL REPORT

STUDY TITLE: **EARTHING EFFECTS IN FEMALE LEWIS RATS**

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Study Initiation Date: June 17, 2008

Study Completion Date: March 4, 2010

SUMMARY

The purpose of this study is to determine whether differences between grounded and ungrounded animals can be found over the life span of female Lewis retired breeder rats, which is about two years. Two groups of 30 rats, one group housed on grounding mats and the other without mats were entered into the study. Blood samples were taken every 4 weeks for 169 days, then every 8 weeks for 14 months and analyzed for clinical chemistry and hematological parameters, and the biomarkers tumor necrosis factor alpha (TNFα), nitrate/nitrite (NO_x) and C-reactive protein (CRP). The resulting data were compared between the treatment groups. At 169 days, significant differences between grounded and ungrounded animals were seen in several parameters. Some could be factors forming a pattern that may be related to metabolic syndrome (TG, GLU, ALP, CRP), others to kidney function (CRE, UREA) and still others to factors affecting serum proteins (TP, GLOB). Some parameters showed progressive changes over time (TG, CRP, NOx and TNFα).

In the final results, most of the small but significant differences between treatment groups observed at the Study Day 169 point in the study persisted to the end of the study. Although the group differences were small and the mean values of the parameters fell mostly in the normal range, two potentially important patterns were observed. The first pattern may be related to a potentially beneficial effect of grounding on metabolic syndrome, in that serum triglycerides, glucose, alkaline phosphatase and also body weight were lower in the grounded group than in the ungrounded group. The other may relate to immune responsiveness, as evidenced by the slightly higher levels of serum globulins and the related parameters total protein and albumin-globulin ratio in the grounded group. Other intergroup differences not falling into a pattern included lower lymphocyte counts, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration and higher segmented neutrophils, absolute segmented neutrophil counts, and platelet counts in the grounded than in the ungrounded group. As well, the grounded group had higher serum creatinine levels, lower serum potassium levels (potentially associated with intracellular effects of grounding), higher serum aspartate transaminase levels (an indicator of liver damage), and lower C-reactive protein at Study Day 169 (changing to higher C-reactive protein over the course of the study) than the ungrounded group. All of the parameter means fell close to the reported normal ranges and none of the statistically significant differences affected survival rates under the conditions of the study.

It was concluded that electrical grounding can subtly affect physiological parameters, and this has the potential to have an impact on the health of an at-risk individual. Further study of the effects of grounding under different experimental conditions may help elucidate the relevance of our observations.

1.0 GENERAL POINTS

1.1 Test Article

Electrical grounding mats connected to building earth.

1.2 Objective

To evaluate the lifetime effects of electrical grounding vs. the ungrounded condition in retired breeder female Lewis rats.

1.3 Schedule of the Study

- Experimental starting date: June 24, 2008
- First day of treatment: June 25, 2008
- Last day of treatment: March 4, 2010
- Day of necropsy: March 4, 2010
- Experimental completion date: March 4, 2010

2.0 TEST ITEM INFORMATION

A stainless steel mesh pad electrically grounded to the building ground, in each standard shoebox cage containing test animals, was used as the test item to determine the effect of electrical grounding. The mesh pads were 415 x 195 mm to cover the bottom of each cage. A 215 mm vertical grounding post was welded to the corner of each mat. A wire lead connected to the building electrical ground was attached to the top of each post with an alligator clip. The mesh was ¼" x ¼" (6.35 x6.35 mm) woven stainless steel, wire size 0.0785" (2 mm). For the protection of the animals, the mesh edges were covered with a 10 mm wide folded 20 gauge (0.9525 mm thick) stainless steel frame. The grounding post was made of 0.125" (3.175 mm) diameter stainless steel rod.

3.0 EXPERIMENT PROCEDURE

3.1 Test System and Environment

3.1.1 ***Species, Strain, Supplier and Specifications***

- Species/Strain: Rat, Lewis, female retired breeders
- Supplier: Charles River Canada Inc
- Number of Animals in the Study: 60
- Age at Initiation of Treatment: 4-6 months
- Sex: Female
- Body Weight Range at Initiation of Treatment: 260.1 g – 372.2 g

3.1.2 ***Environment and Husbandry***

The animal room environment was controlled with targeted conditions:

- Housing: in pairs

- Temperature: 18-26 degrees C.
- Relative Humidity: 30-70%
- Air Changes: 15-20/h
- Light Cycle: 12 h
- Caging: Shoebox cages with environmental enrichment (metal tubes in contact with the grounding pads in grounded group) and some with metal tube only (ungrounded group). In cages with grounding pad, bedding depth was kept minimal so that at least one foot was always touching the pad.

3.1.3 ***Diet and Water***

- Diet: Certified Pico-Rodent chow
- Water: *ad libitum* UV sterilized reverse osmosis water.
- Bedding: Anderson's Bed-O-Cobs, (Diamondsoft when requested by veterinarian)

The study director has reviewed the feed, bedding and water analysis for contaminants and found none present.

3.2 Pre-treatment Procedures

- Animal Health Procedure: Rats were observed daily and body weights taken on the first day of the pretest period. A health status report was generated prior to animals being released to the study.
- Acclimation Period: 7 days
- Allocation to Treatment Group: Rats were allocated with SAS PROC PLAN to minimize differences in body weight between treatment groups.
- Identification of the Animals: tail tattoo
- Selection Criteria: Only animals in apparent good health and within the specified age range were selected for randomization to treatment groups.
- Identification Numbers: Rats were assigned a unique number within 3 days of arrival at the Facility.
- Identification of the Cages: Cage cards

3.3 Treatment

3.3.1 ***Experimental Design***

3.3.1.1 ***Test Treatments***

This study was intended to build upon a model validated previously. The effects of electrical grounding vs. a non-grounded control condition on clinical chemistry, hematology and biomarker parameters were followed for 20 months. A total of 60 rats entered the study. Two groups of 30 animals were each assigned to specific treatments. Group breakdown was as below in Table 1.

Table 1. Treatment Groups for the Study

Group No.	Test Treatment	Purpose
1	Grounded	To evaluate the effects of electrical grounding on various biological indicators
2	None	To establish that the model system was performing as expected

Test treatments involve housing in shoebox cages with electrical grounding mats connected to the room's electrical ground by the Earth FX earthing technology starting on Study Day 1. Induced voltage measurements on the enrichment tubes vs. the building ground were recorded daily.

Animals surviving for the duration of the study were sacrificed at 20 months, at an approximate age of 2 years.

The experimental procedure schedule is summarized in Table 2. Note that the numbers of animals are those based upon initial planning. Actual numbers were subject to attrition as the project progressed and are given in the results section.

Table 2. Experimental Procedure Schedule

Study Day Number	Blood Collection via Jugular	Blood Collection via Abd. Vena Cava & Sacrifice
1	60 animals	
29	60 animals	
57	60 animals	
85	60 animals	
113	60 animals	
141	60 animals	
169	60 animals	
225	60 animals	
281	60 animals	
337	60 animals	
393	60 animals	
449	60 animals	
505	60 animals	
561	60 animals	
618		60 animals

The treatment schedule is summarized in Table 3.

Table 3. Treatment Schedule

Treatment Group	Treatment	No. of Animals per Group
1	Grounded	30
2	None	30

3.3.1.2 ***Observations***

Observations were performed once daily, with full clinical observations once weekly.

3.3.1.3 ***Body Weight***

Animals were weighed on the first pretest day, on Study Day 1 and at each blood collection time and prior to euthanasia.

3.3.1.4 ***Clinical Pathology***

On study day 1 and thereafter according to the study schedule in Table 2 until study day 618, animals were anesthetized with isoflurane and blood was collected from all animals for biomarker, hematological and clinical chemistry analyses as described in the following sections.

3.3.1.5 ***Hematology***

The following parameters were evaluated on an Abbott Cell-Dyn® 3700 CS using Abbott reagents:

- Red blood cell count (RBC) and morphology (morphology performed manually)
- White blood cell count (WBC)
- Absolute and relative differential white blood cell count (manual: segmented neutrophils, SEG; absolute segmented neutrophils, ABS SEG; band neutrophils, BAND; absolute band neutrophils, ABS BAND; lymphocytes, LYMPH; absolute lymphocytes, ABS LYMPH; monocytes, MONO; absolute monocytes, ABS MONO; eosinophils, EOS; absolute eosinophils, ABS EOS; basophils, BASO; absolute basophils, ABS BASO)
- Hematocrit (HCT)
- Hemoglobin (HGB)
- Mean Cell Hemoglobin (MCH)
- Mean Cell Volume (MCV)
- Mean Cell Hemoglobin Concentration (MCHC)
- Platelets (PLT)

3.3.1.6 ***Biomarkers***

The following biomarkers were evaluated via ELISA methods:

- C-reactive protein (CRP), Helica Biosystems, Inc.
- Tumor necrosis factor alpha (TNFα), Biosource/Invitrogen Corp.
- Nitric oxide (Serum nitrite/nitrate, NOx), OXIS International, Inc.

3.3.1.7 ***Clinical Chemistry***

The parameters in Table 4 were evaluated:

Table 1. Parameters Evaluated on the Hitachi 912 Automatic Analyzer

Parameter Analyzed	Method of Analysis	Reagent Manufacturer
Alanine Aminotransferase (ALT)	IFCC without pyridoxal (UV test)	Roche
Albumin (ALB)	Bromocresol green	Roche
Albumin/Globulin Ratio (AG Ratio)	Calculated	N/A
Alkaline Phosphatase (ALP)	ALP-IFCC liquid	Roche
Aspartate Aminotransferase (AST)	IFCC without pyridoxal	Roche
Calcium (CA)	o-Cresolphtalein complexone	Roche
Chloride (CL)	Ion Selective Electrode with dilution	Roche
Cholesterol (CHOL)	CHOD-PAP (enzymatic colorimetric)	Roche
Creatinine Kinase (CK)	CK-liquid IFCC	Roche
Creatinine (CRE)	Jaffe (kinetic) rate-blanked and compensated	Roche
Globulin (GLOB)	Calculated (total protein – albumin)	N/A
Glucose (GLU)	GOD-PAP	Roche
Sorbitol dehydrogenase (SDH)	SDH-oxidation of NADH (UV)	Catachem
Phosphorus (PHOS)	Molybdate	Roche
Potassium (K)	Ion Selective Electrode with dilution	Roche
Sodium (NA)	Ion Selective Electrode with dilution	Roche
Total Bilirubin (TBIL)	Jendrassik	Roche
Total Protein (TP)	Biuret	Roche
Triglycerides TG)	GPO-PAP (enzymatic colorimetric)	Roche
Urea Nitrogen (UREA)	Urea kinetic (UV)	Roche

Other calculated parameters include:

- Lipemic Index (L)
- Hemoglobin Index (H)
- Icteric Index (I)

3.3.1.8 ***Induced Voltage***

The induced voltage on the metal enrichment tubes in both groups was recorded daily, thereby providing an estimate of the electric field exposure to animals in the two groups.

3.3.2 ***Euthanasia***

Euthanasia was conducted via abdominal vena cava bleed under isoflurane anesthesia.

3.3.3 ***Necropsy***

Rats found dead or undergoing unscheduled necropsy were subjected to blood and tissue collection at the discretion of the study director or veterinarian conducting the necropsy, in order to establish diagnosis of any detectable pathologies.

All remaining rats were euthanized by exsanguination under anesthesia on terminal study day 617 or 618, during final blood collections. The following tissues were examined and collected into

10% neutral buffered formalin or other fixative to observe any sub-clinical inflammatory response. Additional tissues were taken at the discretion of the study director or pathologist.

- cervical/mandibular, mesenteric, iliac lymph nodes
- spleen
- liver
- lung
- kidney

3.3.4 ***Histology***

The collected tissues listed above were sectioned and stained, along with any other tissues taken. The veterinary pathologist examined the slides and reported his findings.

4.0 DATA EVALUATION

- Data analysis followed a two-way analysis of variance (ANOVA, group by study day) for normally distributed data and an equivalent non parametric procedure for non-normal data.
- Statistical analyses were performed using SAS Release 9.2 for Windows XP. Statistical procedures were selected based on the distribution of the data and the validity of the assumptions.
- Statistical significance was declared when $p \leq 0.05$.
- Base levels for all parameters were obtained on Test Day 1 before beginning treatments.
- Body weight data was analyzed using repeated measures ANOVA with SDAY1 (Study Day 1) measurements as covariate. The model included treatment group (1 and 2), study day (29, 57, 85, 113, 141, 169, 197, 225, 253, 281, 337, 393, 450, 506, 561, and 618) and treatment group by study day interaction as fixed effects.
- Clinical chemistry, CRP biomarker and hematology parameters were analyzed using a repeated measures analysis of variance. The model included treatment group (group 1 and 2), study day (29, 57, 85, 113, 141, 169, 197, 225, 253, 281, 337, 393, 450, 506, 561, and 618) and treatment group by study day interaction as fixed effects as well as the pre-dose parameter value as a covariate. The exceptions to this were TBIL, BASO, ABS-BASO, BAND, and ABS BAND which were analyzed using the Cochran – Mantel – Haenszel's row mean score statistic due to low variability in the data while controlling for study days..
- A natural log transformation was used for CK, SDH, AST, ALT, ALP, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, WBC, SEG, ABS SEG, LYMPH, ABS LYMPH, MONO, ABS MONO, EOS, ABS EOS and CRP in an attempt to normalize the data. A number of the parameters had outlying observations, which were investigated in terms of the impact they had on the results. Outliers were defined by observations that were greater than 3 standard deviations away from the means.
- MCHC and PLT were analyzed using a non-parametric ANOVA since they were not normally distributed.
- Group variances were tested for homoscedasticity using the Brown and Forsythe test for normally distributed data or the Ansari-Bradley test for non-normal data. Significant departure from equality of variance was declared when $P < 0.05$ and the model modified to

correct the difference. Differences were observed between groups for hematology parameters, as well as CRP. The model used took into account these differences.

- For statistical analysis of body weight, clinical chemistry and hematology parameters and CRP, if the model revealed statistical significance ($p \leq 0.05$), Tukey-Kramer adjusted comparisons were used to determine if pairwise group differences existed.
- Kaplan-Meier (non-parametric maximum likelihood) method was used to estimate the survival curves for the two groups. Survival was defined by the length of time the animal remained in the study. An animal was removed from the study on the day it was euthanized or found dead. The log-rank test was used to compare the survival curves between the two groups. Fisher's exact test was used to compare the types of diagnoses between groups.

5.0 RESULTS

The text of this section focuses mainly on parameters for which statistical and biological differences between groups were found.

5.1 Electric Field Measurements

Daily induced voltage measurements on the enrichment tubes vs. the building ground were all 0 in the grounded group, while in the ungrounded group, the daily measurements averaged 39.6 ± 18.1 mVAC (millivolts alternating current), with a range from 8.9-268.0 mVAC.

5.2 Mortality

As this was a study of long duration covering the average life span of a rat, attrition was an expected feature of the study. As the study progressed, increasing numbers of animals died or were euthanized with terminal conditions. Therefore, survival analysis of the mortality data was undertaken. The study day on which the animal was either found dead or was euthanized was used to represent the survival time. The statistician notes that caution should therefore be used in the interpretation since euthanized animals would likely have survived beyond the time points at which they were euthanized. Nevertheless, the criteria for euthanasia were the same for both groups so the data are comparable.

The survival curves for the two groups over the course of the 618 days of study are shown in Figure 1. The two curves did not differ significantly (p=0.0632), although there was a tendency for ungrounded animals to last in the study longer than grounded animals.

Interpretation: Electrical grounding did not result in significantly increased longevity of retired breeder female Lewis rats, when compared with ungrounded animals.

5.3 Clinical Observations

The clinical observations present the first view of underlying pathologies as the animals progressed through their lives, and gave the investigators clues that led to definitive diagnoses on necropsy. For instance, masses were observed as they arose, grew, and reached sufficient size to trigger humane

euthanasia. The location of the masses in for instance the mammary tissue or lymph nodes was directly associated with the eventual diagnosis. Neurological signs such as head tilt, circling or ataxia were signs of a central nervous system disorder, and invariably led to the finding of a pituitary mass.

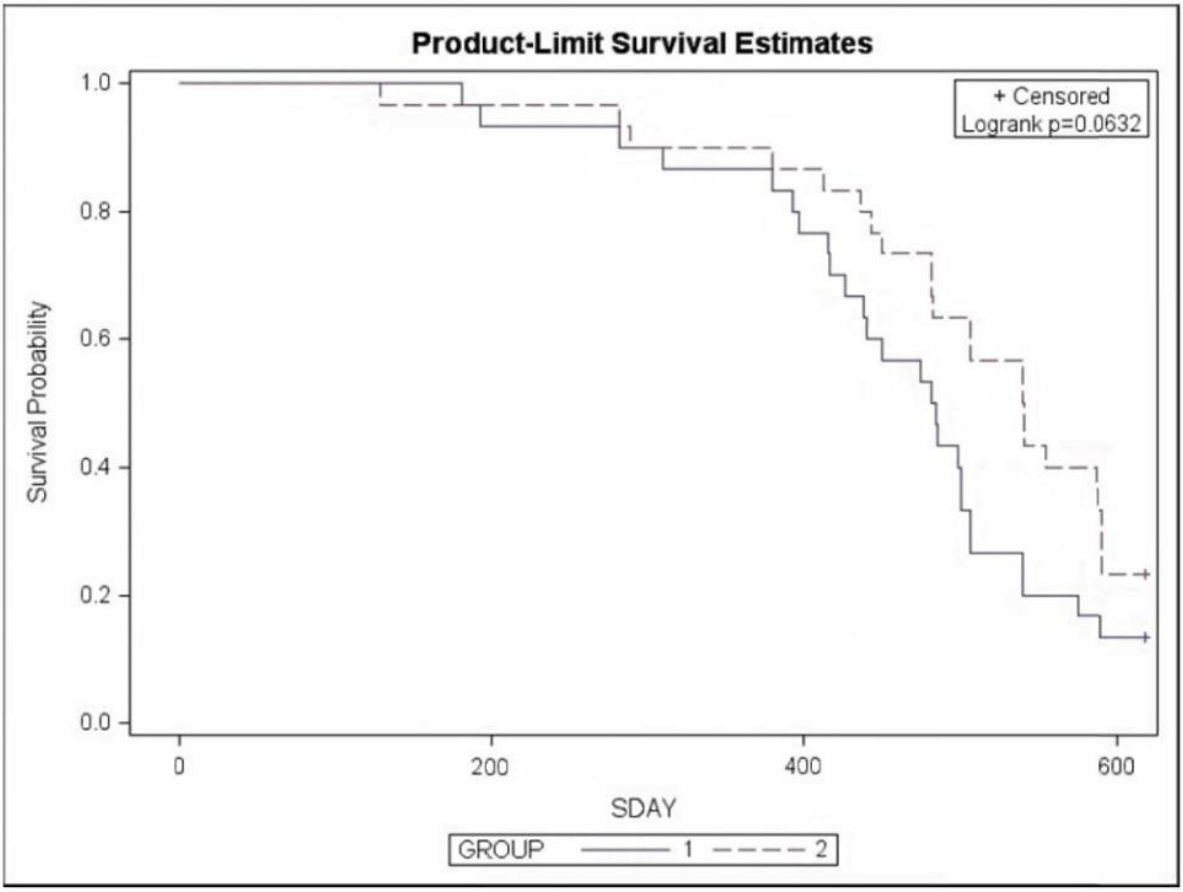

Figure 1. Survival curves for Grounded (Group 1) and Ungrounded (Group 2) rats.

Interpretation: All of the clinical signs observed, in keeping with the findings of the veterinary pathologist, could be associated with conditions seen regularly in aging rats. These are covered in Section 5.7.2. The one exception would be the hind foot lesions seen in grounded Rats numbers 8, 10, 11, 15, 20, 27, 43, 50, 52, and 55, which varied from mild redness through swelling, and ulceration of the pads. These superficial and localized lesions were attributed to direct mechanical irritation from the wire grounding pads that occurred despite the use of extra bedding and changes to softer bedding. It is unlikely that these lesions would have affected any of the parameters measured in this study due to their localized nature.

5.4 <u>Body Weights</u>

In the analysis of body weights, group ($p=0.222$) study day ($p<0.0001$), group by study day interaction ($p=0.0013$) and the pre-dose weight covariate ($p<0.0001$) were all significant in the model. The significance of the pre-dose weight covariate in the model implies a significant correlation between the rodents' pre-dose body weight and the rodents' body weights during the course of the study. Group 1 had

significantly lower body weights than Group 2 (p=0.0222; Table 5). Body weights significantly increased over the course of the study up to study day 450. At this point, body weights on animals remaining in the study were on a decreasing trend. For study days 29, 57, 85, 113, 141, 169, 197, 225, 253, 281, 337, 393, 561, and 618, Groups 1 and 2 did not differ significantly in terms of body weights (all p>0.05). Group 1 had significantly lower body weights than Group 2 for study days 450 (p=0.0049) and 506 (p=0.0465). Sample sizes dropped off significantly from study day 450 on as animals were either found dead or were euthanized.

Table 5. Summary Statistics for Body Weight (g) by Group and Study day

	Grounded (Group 1)			Not Grounded (Group 2)		
Study Day	N	Mean	SD	N	Mean	SD
1	30	301.25	16.56	30	304.95	22.63
29	30	312.24	19.34	30	319.17	26.82
57	30	319.51	18.49	30	329.01	30.27
85	30	326.41	21.79	30	331.15	32.16
113	30	330.62	23.04	30	339.72	34.51
141	30	341.06	24.10	29	353.55	38.87
169	30	349.46	26.61	29	362.50	39.05
197	28	370.68	29.07	29	381.97	42.12
225	28	366.54	28.14	29	381.31	41.74
253	28	391.51	32.84	29	404.43	44.33
281	28	398.03	34.08	29	412.94	46.99
337	26	422.41	33.82	27	434.58	39.36
393	25	429.67	38.81	26	447.41	38.40
450	18	435.89	45.01	23	473.85	41.87
506	10	449.02	53.20	19	463.74	36.73
561	6	430.08	56.61	12	453.88	44.23
618	4	413.93	29.25	7	459.27	80.23

The body weights for both groups over time are plotted in Figure 2.

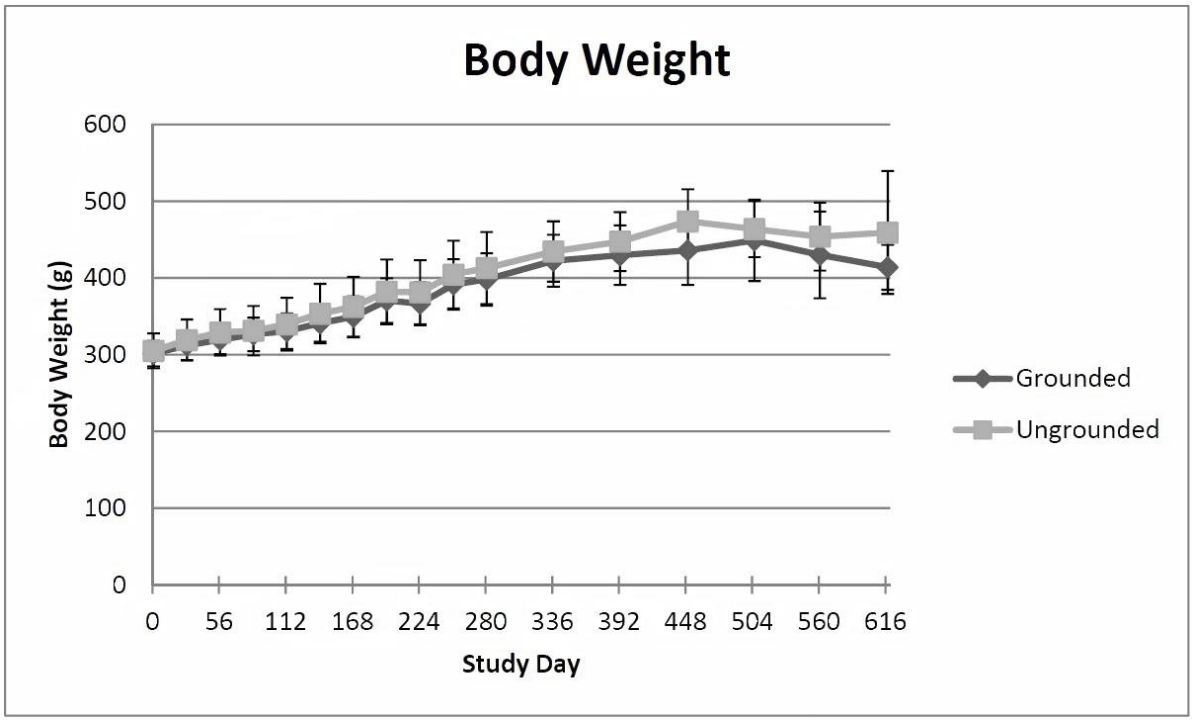

Figure 2. Mean ± SD body weights for both groups over the course of the study.

Interpretation: There appeared to be no treatment effects of grounding on body weight during the first 169 days. Overall, animals in both groups gained weight over time, as would be expected. The trend toward decreasing body weight after study day 450 is difficult to interpret as at the same time, sample sizes dropped off significantly as animals were either found dead or were euthanized. The overall group differences and the data from study days 450 and 506 are consistent with electrical grounding causing slightly lower growth rate in the study animals. There was a trend toward divergence of the two groups over the course of the study. Therefore, the data are consistent with a conclusion that electrical grounding slightly lowers body weight. The possible causes for this will be discussed in context with the other significant group differences in the data in the conclusion section.

5.5 <u>Hematology</u>

MCH, MCHC, SEG, ABS SEG, LYMPH, and ABS LYMPH showed significant group differences (Tables 6 and 7). Table 8 presents differences between groups for all hematology parameters on Study Day 1.

Table 6. Summary Statistics for Hematology Parameters by Group and Study Day.

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)
RBC	Mean	8.19	8.74	8.91	8.49	8.63	8.32[10]	8.80	9.02	8.41	8.43
	SD	0.43	0.34	0.31	0.28	0.31	0.35	0.41	0.36	0.34	1.18
HGB	Mean	143.53	157.83	158.50	152.67	156.53	145.97[10]	158.97	160.20	151.73	154.53
	SD	6.82	6.14	4.54	3.85	4.64	5.42	7.04	6.17	6.34	15.80
HCT	Mean	0.41	0.45	0.45	0.44	0.45	0.41[10]	0.45	0.45	0.43	0.44
	SD	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.05
MCV	Mean	49.83	51.40	50.35	52.29	51.98	49.29[10]	51.17	50.30	51.70	52.70
	SD	1.05	0.83	0.73	0.78	1.03	0.90	0.52	0.52	0.66	6.20
MCH	Mean	17.53	18.05	17.80	18.00	18.14	17.56[10]	18.06	17.77	18.04	18.67
	SD	0.43	0.25	0.29	0.33	0.26	0.28	0.19	0.21	0.22	2.57
MCHC	Mean	351.87	351.17	353.57	344.20	349.13	356.28[10]	353.03	353.47	348.93	353.57
	SD	4.24	4.81	4.23	5.40	4.87	3.32	3.23	2.97	4.78	5.71
PLT	Mean	594.70[1]	588.00[2]	565.96[3]	599.07[4]	608.62[5]	627.21[10]	598.25[6]	547.63[7]	593.60[8]	551.24[9]
	SD	102.42	111.23	79.87	90.78	61.13	62.45	89.41	115.62	68.48	108.59
WBC	Mean	4.11	4.71	4.84	4.19	4.50	4.35[10]	4.60	4.83	4.14	4.20
	SD	1.02	0.75	0.97	0.97	1.01	0.94	0.92	0.93	1.16	0.95
SEG	Mean	0.26	0.28	0.27	0.30	0.29	0.22	0.23	0.24	0.29	0.26
	SD	0.05	0.07	0.06	0.07	0.08	0.05	0.06	0.06	0.06	0.05
ABS SEG	Mean	1.08	1.31	1.33	1.27	1.35	0.95[10]	1.09	1.16	1.25	1.09
	SD	0.38	0.46	0.52	0.51	0.62	0.28	0.48	0.38	0.60	0.31

Table 6 continued…

Parameter	Study Day	Grounded (Group 1) 1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)	Not Grounded (Group 2) 1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)
BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00[10]	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00
LYMPH	Mean	0.69	0.68	0.70	0.68	0.68	0.72	0.72	0.72	0.68	0.70
	SD	0.05	0.08	0.07	0.07	0.08	0.05	0.06	0.06	0.06	0.05
ABS LYMPH	Mean	2.80	3.19	3.36	2.82	3.02	3.15[10]	3.31	3.49	2.80	2.96
	SD	0.67	0.59	0.62	0.65	0.58	0.75	0.60	0.70	0.65	0.71
MONO	Mean	0.05	0.04	0.03	0.02	0.02	0.05	0.04	0.03	0.02	0.03
	SD	0.02	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.01	0.02
ABS MONO	Mean	0.21	0.21	0.13	0.08	0.10	0.22[10]	0.17	0.15	0.07	0.13
	SD	0.11	0.11	0.12	0.06	0.08	0.10	0.09	0.10	0.05	0.07
EOS	Mean	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01
	SD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ABS EOS	Mean	0.02	0.01	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02
	SD	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.03	0.02	0.02
BASO	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BASO	Mean	0.00	0.00	0.00	0.00	0.00	0.00[10]	0.00	0.00	0.00	0.00
	SD	0.01	0.00	0.01	0.01	0.01	0.00	0.01	0.01	0.01	0.01

[1] IDNO 44, 49 and 50 missing PLT therefore n=27. [2] IDNO 20 and 49 missing PLT therefore n=28.
[3] IDNO 10, 14, 33 and 53 missing PLT therefore n=26. [4] IDNO 1, 4 and 15 missing PLT therefore n=27.
[5] IDNO 24 missing PLT therefore n=29. [6] IDNO 31 and 60 missing PLT therefore n=27.
[7] IDNO 22, 23, 25, 47, 51 and 60 missing PLT therefore n=24.
[8] IDNO18, 22, 41, 51 and 60 missing PLT therefore n=25.
[9] IDNO 51 missing PLT therefore n=29. [10] IDNO 3 sample clotted therefore only able to get SEG, LYMPH, MONO, EOS, BASO and BAND therefore n=29 for remaining parameters.

Table 6 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	141 (n=30)	169 (n=30)	225 (n=28)	281 (n=28)	337 (n=26)	141 (n=29)	169 (n=29)	225 (n=29)	281 (n=29)	337 (n=27)
RBC	Mean	8.30	8.55	8.33	8.57	8.58	8.46	8.63	8.23	8.47	8.39
	SD	0.30	0.29	0.58	0.27	0.40	0.23	0.30	0.97	0.41	0.45
HGB	Mean	152.40	157.60	154.71	167.07	167.65	155.14	159.55	152.86	166.17	164.56
	SD	4.77	4.11	8.25	5.09	7.40	4.63	4.74	15.77	6.89	7.15
HCT	Mean	0.45	0.45	0.44	0.45	0.46	0.45	0.46	0.44	0.45	0.45
	SD	0.01	0.01	0.03	0.01	0.02	0.01	0.01	0.04	0.02	0.02
MCV	Mean	53.72	52.87	53.29	52.84	53.18	53.23	52.86	53.39	53.02	53.35
	SD	0.94	0.88	0.77	0.75	0.90	0.90	0.77	2.14	1.08	1.24
MCH	Mean	18.36	18.44	18.62	19.50	19.55	18.35	18.48	18.64	19.63	19.63
	SD	0.53	0.32	0.92	0.30	0.41	0.59	0.28	0.68	0.41	0.54
MCHC	Mean	341.90	348.80	349.43	368.93	367.42	344.79	349.79	349.10	370.41	367.85
	SD	8.36	3.48	16.80	5.25	5.83	10.47	3.44	4.92	6.43	6.38
PLT	Mean	614.55[1]	632.37	591.61	599.78[2]	583.15	614.11[3]	628.07	592.90	580.14[4]	551.12[5]
	SD	79.43	45.77	61.13	57.16	69.60	78.77	64.28	60.54	63.72	64.35
WBC	Mean	3.68	4.22	4.66	4.49	4.64	3.83	4.48	4.82	4.45	4.40
	SD	0.57	0.65	0.82	0.90	0.89	0.78	1.05	0.87	0.91	1.05
SEG	Mean	0.30	0.32	0.31	0.33	0.34	0.27	0.28	0.28	0.30	0.32
	SD	0.07	0.05	0.07	0.04	0.08	0.06	0.04	0.04	0.05	0.06
ABS SEG	Mean	1.10	1.35	1.44	1.49	1.56	1.04	1.26	1.37	1.34	1.44
	SD	0.35	0.32	0.43	0.36	0.46	0.41	0.39	0.38	0.43	0.51
BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00

Table 6 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	141 (n=30)	169 (n=30)	225 (n=28)	281 (n=28)	337 (n=26)	141 (n=29)	169 (n=29)	225 (n=29)	281 (n=29)	337 (n=27)
LYMPH	Mean	0.67	0.65	0.66	0.63	0.64	0.70	0.69	0.68	0.66	0.65
	SD	0.07	0.06	0.07	0.05	0.08	0.06	0.05	0.05	0.06	0.06
ABS LYMPH	Mean	2.46	2.73	3.05	2.83	2.95	2.66	3.08	3.25	2.93	2.82
	SD	0.36	0.46	0.59	0.58	0.62	0.48	0.70	0.57	0.55	0.65
MONO	Mean	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.03	0.03	0.02
	SD	0.01	0.02	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
ABS MONO	Mean	0.09	0.12	0.14	0.14	0.09	0.10	0.11	0.17	0.14	0.10
	SD	0.05	0.07	0.08	0.08	0.08	0.05	0.07	0.09	0.08	0.06
EOS	Mean	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	SD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.01
ABS EOS	Mean	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.04
	SD	0.02	0.02	0.03	0.03	0.03	0.02	0.03	0.04	0.02	0.03
BASO	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BASO	Mean	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
	SD	0.01	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00

[1] IDNO 37 missing PLT therefore n=29. [2] IDNO 55 missing PLT therefore n=27. [3] IDNO 30 and 47 missing PLT therefore n=27. [4] IDNO 19 missing PLT therefore n=28. [5] IDNO 66 missing PLT therefore n=26.

Table 6 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	393 (n=25)	450 (n=18)	506 (n=10)	561 (n=6)	618 (n=4)	393 (n=26)	450 (n=23)	506 (n=19)	561 (n=12)	618 (n=6)
RBC	Mean	8.75	8.80	8.72	9.63	8.40	8.81	8.72	8.98	8.66	8.83
	SD	0.75	0.77	0.65	0.32	1.62	0.50	0.63	0.44	1.42	0.24
HGB	Mean	161.36	161.61	162.90	176.50	152.25	163.04	163.09	167.47	161.50	163.67
	SD	10.36	13.68	7.82	5.47	30.37	7.89	9.48	6.52	21.25	5.39
HCT	Mean	0.46	0.46	0.46	0.51	0.44	0.46	0.46	0.47	0.46	0.46
	SD	0.03	0.04	0.02	0.01	0.08	0.02	0.03	0.02	0.06	0.01
MCV	Mean	52.61	52.08	53.40	52.45	52.83	52.06	52.28	52.88	53.82	52.62
	SD	2.32	0.86	1.87	0.85	1.14	1.38	1.46	1.66	3.58	1.05
MCH	Mean	18.50	18.39	18.72	18.32	18.13	18.52	18.72	18.67	18.83	18.55
	SD	0.74	0.33	0.67	0.38	0.15	0.42	0.51	0.64	1.23	0.37
MCHC	Mean	351.84	352.94	350.60	349.33	343.00	355.77	358.22	352.89	350.17	352.67
	SD	4.08	4.49	4.27	4.03	9.45	3.18	4.19	4.40	2.55	2.58
PLT	Mean	601.26[1]	582.83	617.70	541.83	578.25	555.30[2]	537.59[3]	518.32	548.75	541.17
	SD	71.02	93.25	142.20	46.61	83.85	67.65	122.75	131.01	59.23	89.51
WBC	Mean	5.04	5.11	6.42	5.88	5.39	5.00	4.89	4.38	5.73	4.90
	SD	1.17	1.71	3.19	1.46	1.23	1.81	1.35	0.86	1.76	0.92
SEG	Mean	0.37	0.38	0.37	0.42	0.43	0.32	0.34	0.36	0.38	0.41[4]
	SD	0.08	0.09	0.09	0.08	0.08	0.07	0.07	0.07	0.09	0.10
ABS SEG	Mean	1.92	1.91	2.59	2.46	2.40	1.68	1.71	1.60	2.25	2.13
	SD	0.70	0.81	1.96	0.71	0.95	1.11	0.74	0.48	1.01	0.83
BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00[4]
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BAND	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00

Table 6 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	393 (n=25)	450 (n=18)	506 (n=10)	561 (n=6)	618 (n=4)	393 (n=26)	450 (n=23)	506 (n=19)	561 (n=12)	618 (n=6)
LYMPH	Mean	0.60	0.59	0.59	0.54	0.53	0.65	0.63	0.60	0.59	0.57[4]
	SD	0.08	0.08	0.09	0.08	0.08	0.08	0.07	0.07	0.10	0.10
ABS LYMPH	Mean	2.99	3.02	3.60	3.22	2.79	3.18	3.05	2.63	3.31	2.64
	SD	0.64	1.17	1.21	1.03	0.47	0.80	0.76	0.53	0.91	0.40
MONO	Mean	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.02[4]
	SD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
ABS MONO	Mean	0.09	0.15	0.20	0.16	0.17	0.10	0.10	0.11	0.15	0.10
	SD	0.06	0.10	0.11	0.09	0.05	0.08	0.06	0.06	0.08	0.03
EOS	Mean	0.01	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.00	0.00[4]
	SD	0.01	0.00	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.00
ABS EOS	Mean	0.04	0.02	0.02	0.05	0.03	0.03	0.03	0.03	0.02	0.02
	SD	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.02
BASO	Mean	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00[4]
	SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
ABS BASO	Mean	0.01	0.01	0.02	0.00	0.00	0.00	0.00	0.01	0.00	0.01
	SD	0.01	0.01	0.03	0.00	0.00	0.01	0.01	0.01	0.00	0.01

[1] IDNO 24 and 40 missing PLT therefore n=23, [2] IDNO 25, 42 and 62 missing PLT therefore n=23,
[3] IDNO 13 missing platelet therefore n=22, [4] IDNO 45 sample clotted only SEG, LYMPH, MONO, EOS, BASO and BAND therefore n=7.

Table 7. Results of Statistical Analysis of Hematology Parameters.

Parameter	Effect	Significance Level (p-value)	Summary of Significant Group Differences
RBC[1]	Group	0.2916	
	Study Day	<0.0001	
	Group by Study Day	0.1014	
	Predose Covariate	0.5540	
HGB[1]	Group	0.8897	
	Study Day	<0.0001	
	Group by Study Day	0.0373	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.9471	
HCT[1]	Group	0.2209	
	Study Day	<0.0001	
	Group by Study Day	0.0184	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.7355	
MCV[1]	Group	0.2837	
	Study Day	<0.0001	
	Group by Study Day	0.3695	
	Predose Covariate	0.0045	
MCH[2]	Group	0.0242	Group 1 had sig. lower MCH than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.6731	
	Predose Covariate	0.0675	
MCHC[1]	Group	<0.0001	Group 1 had sig. lower MCHC than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.1855	
	Predose Covariate	0.7483	
PLT[1]	Group	0.0105	Group 1 had sig. higher PLT than Group 2.
	Study Day	0.0580	
	Group by Study Day	0.1996	
	Predose Covariate	0.0003	
WBC	Group	0.0772	
	Study Day	<0.0001	
	Group by Study Day	0.0796	
	Predose Covariate	0.3296	
SEG	Group	0.0044	Group 1 had sig. higher SEG than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.7386	
	Predose Covariate	0.0293	
ABS SEG[1]	Group	0.0096	Group 1 had sig. higher ABS SEG than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.4383	
	Predose Covariate	0.0853	

[1] No change in conclusions when outliers were removed.
[2] When outliers were removed, there was no significant group difference for MCH (p=0.0891).

Table 7 continued…

Parameter	Effect	Significance Level (p-value)	Summary of Significant Group Differences
LYMPH	Group	0.0060	Group 1 had sig. lower LYMPH than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.7565	
	Predose Covariate	0.0086	
ABS LYMPH[2]	Group	0.6763	
	Study Day	<0.0001	
	Group by Study Day	0.0403	Group 1 had sig. higher ABS LYPMH than Group 2 on sday 506 (p=0.0476).
	Predose Covariate	0.4764	
MONO[1]	Group	0.6624	
	Study Day	<0.0001	
	Group by Study Day	0.0719	
	Predose Covariate	0.0546	
ABS MONO[1]	Group	0.1500	
	Study Day	<0.0001	
	Group by Study Day	0.0530	
	Predose Covariate	0.1848	
EOS[1]	Group	0.6937	
	Study Day	0.1019	
	Group by Study Day	0.3824	
	Predose Covariate	0.0085	
ABS EOS[1]	Group	0.7897	
	Study Day	0.0832	
	Group by Study Day	0.4254	
	Predose Covariate	0.0021	
BASO	Group	0.9320	
ABS BASO	Group	0.6267	
BAND	Group	0.1215	
ABS BAND	Group	0.2473	

[1] No change in conclusions when outliers were removed.
[2] When outliers removed, group by study day interaction was not significant (p=0.4410).

Table 8. Differences between groups for hematology parameters on Study Day 1

Parameter	Sex	Sample Size	EFFECT	Significance Level for Group (p-value)	Interpretation of Significant Findings‡*
ABS LYMPH	F	59	GROUP	0.0225	GROUNDED Signif. LT NOT-GROUNDED
LYMPH	F	59	GROUP	0.0240	GROUNDED Signif. LT NOT-GROUNDED
ABS SEG	F	59	GROUP	0.6186	
SEG	F	59	GROUP	0.0110	GROUNDED Signif. GT NOT-GROUNDED
WBC	F	59	GROUP	0.1082	
MCH	F	59	GROUP	0.8196	
MCV	F	59	GROUP	0.0493	GROUNDED Signif. GT NOT-GROUNDED
HCT	F	59	GROUP	0.4846	
HGB	F	59	GROUP	0.1148	
RBC	F	59	GROUP	0.1680	
MCHC‡	F	59	GROUP	<0.0001	GROUNDED Signif. LT NOT-GROUNDED
MONO§	F	59	GROUP	0.8592	
ABS EOS§	F	59	GROUP	0.2747	
ABS MONO§	F	59	GROUP	0.3730	
PLT‡	F	59	GROUP	0.6669	
EOS*	F	60	GROUP	0.9172	
BASO*	F	60	GROUP	0.4915	
ABS BASO*	F	59	GROUP	0.1551	
WBC SCORE*	F	60	GROUP	0.7386	
BAND*	F	60	GROUP		All zeros.
ABS BAND*	F	59	GROUP		All zeros.

§ ANOVA performed on Log transformed values.
‡ The Wilcoxon rank sum test was used for analysis.
* The Cochran – Mantel – Haenszel row mean score statistic, Chi-square test, Fisher's exact tests used.
SDAY – Study Day; GT – Greater Than; Signif – Significantly;

There were the following significant differences between groups in terms of hematology parameters on Study Day 1:

- LYMPH, ABS LYMPH and MCHC were significantly less in the grounded than in the ungrounded group.
- SEG and MCV were higher in the grounded than in the ungrounded group.

There were the following significant differences between groups in terms of hematology parameters across study days (The effects of differences that appeared on Study Day 1 are remove in the statistical analysis):

- LYMPH, MCHC (both less at the Study Day 169 milestone), and MCH were significantly less in the grounded than in the ungrounded group.
- SEG, ABS SEG (both greater at the Study Day 169 milestone), PLT (only higher on Study Day 113 at the Day 169 milestone) and ABS LYMPH (only at Study Day 506) were higher in the grounded than in the ungrounded group. While MCV was also higher at Day 169, this relationship did not hold for the duration of the study.

Graphs showing LYMPH (Figure 3), MCH (Figure 4), MCHC (Figure 5), SEG (Figure 6), ABS SEG (Figure 7) and PLT (Figure 8) over the course of the study are found below.

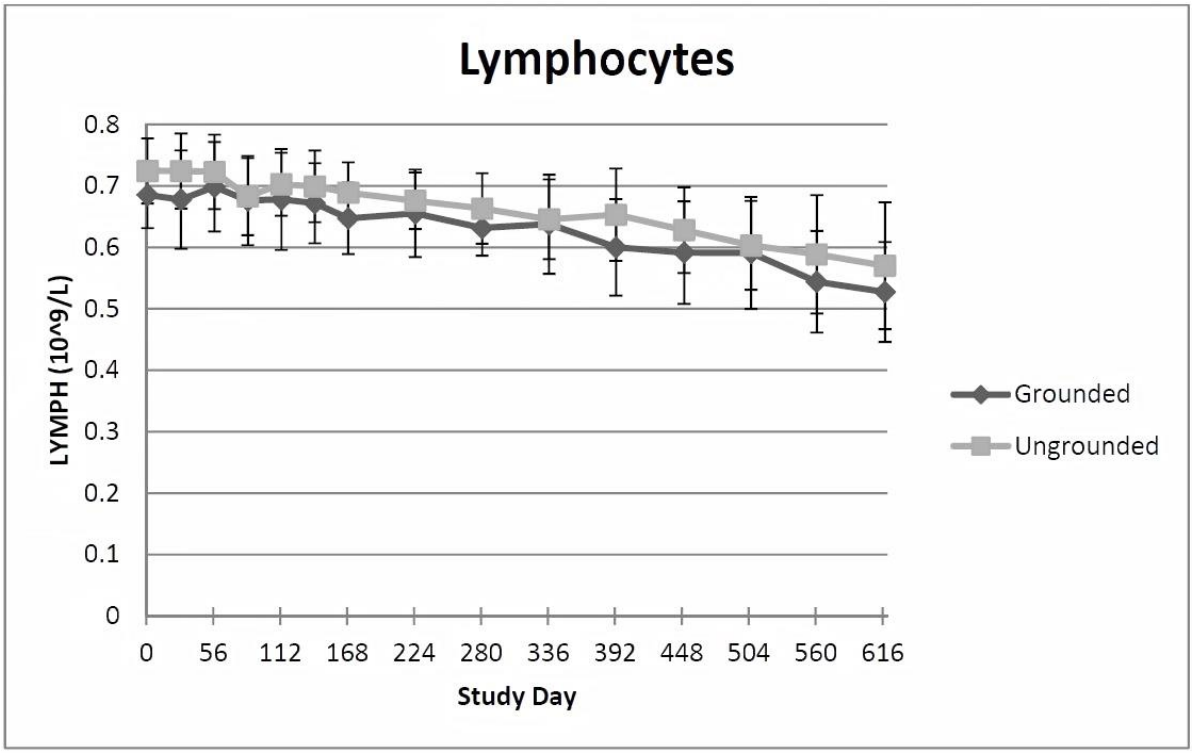

Figure 3. Mean ± SD lymphocyte (LYMPH) counts over the course of the study.

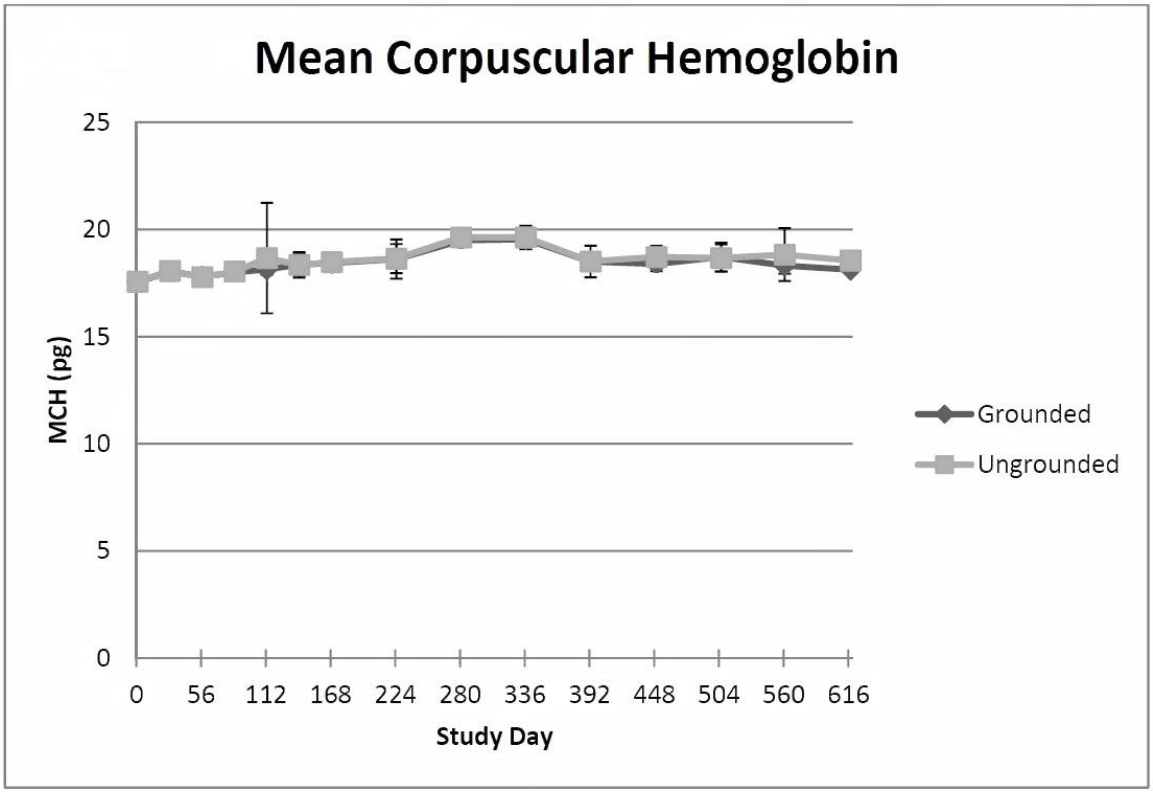

Figure 4. Mean ± SD mean corpuscular hemoglobin (MCH) levels over the course of the study.

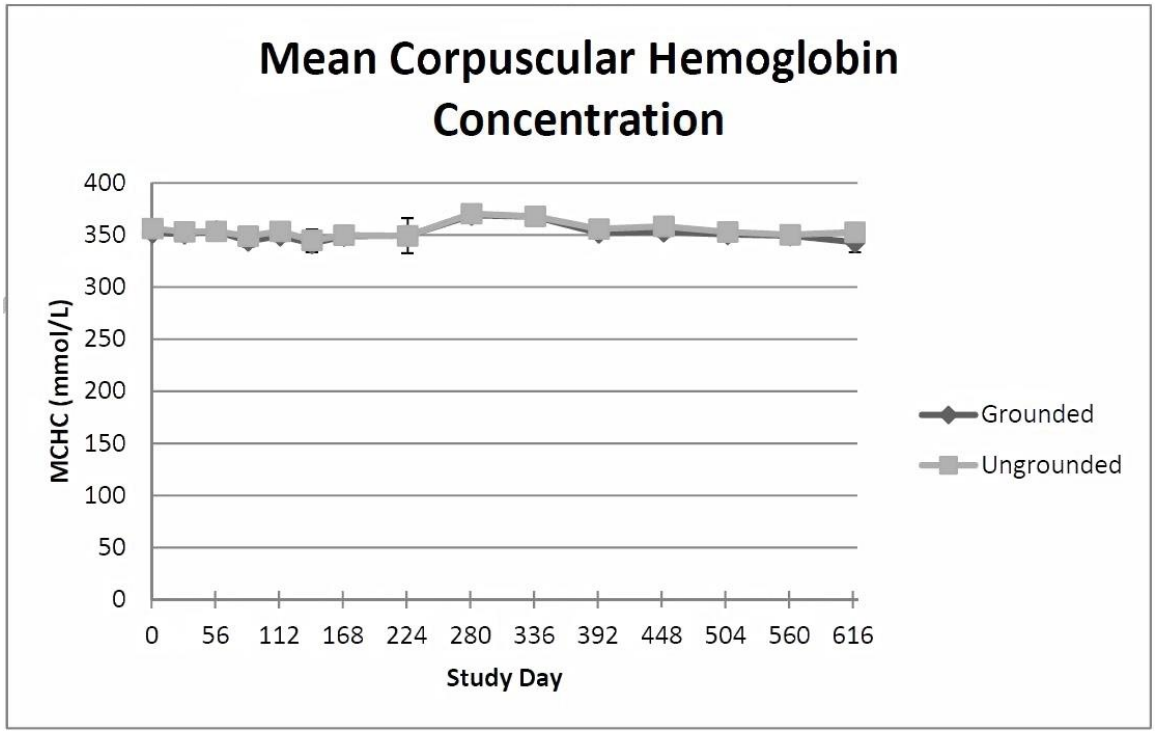

Figure 5. Mean ± SD mean corpuscular hemoglobin concentration (MCHC) over the course of the study.

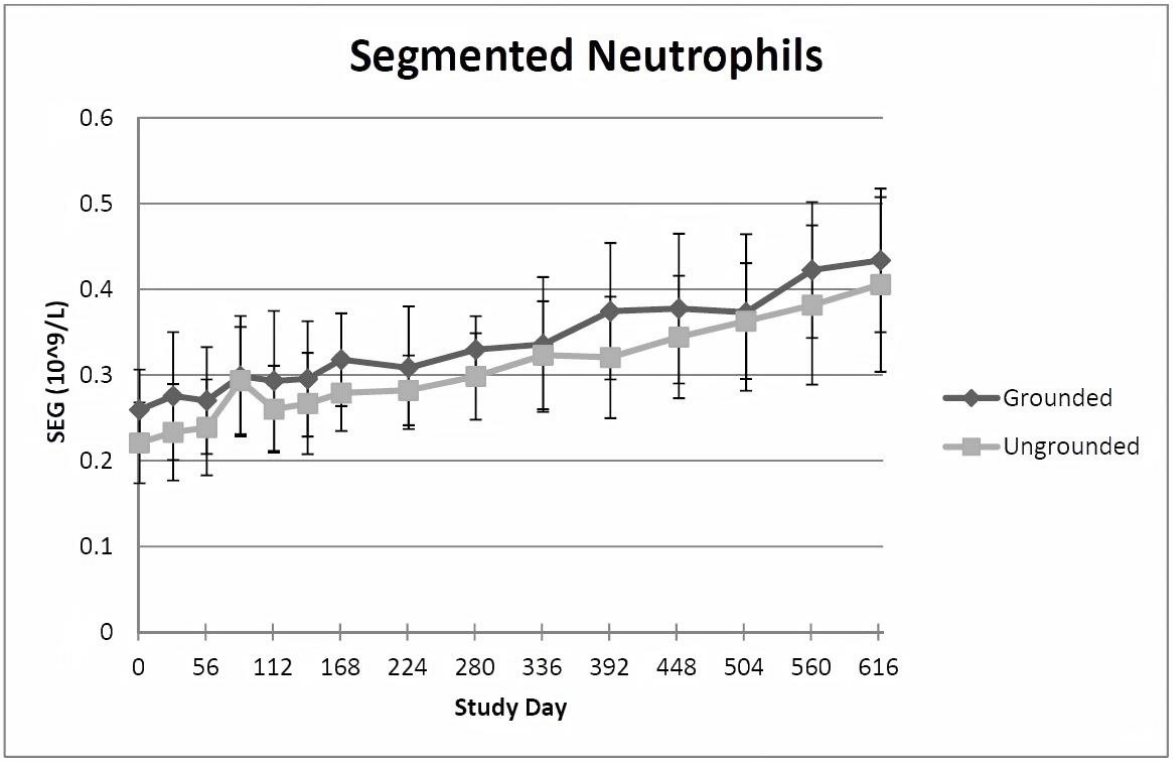

Figure 6. Mean ± SD segmented neutrophil (SEG) counts over the course of the study.

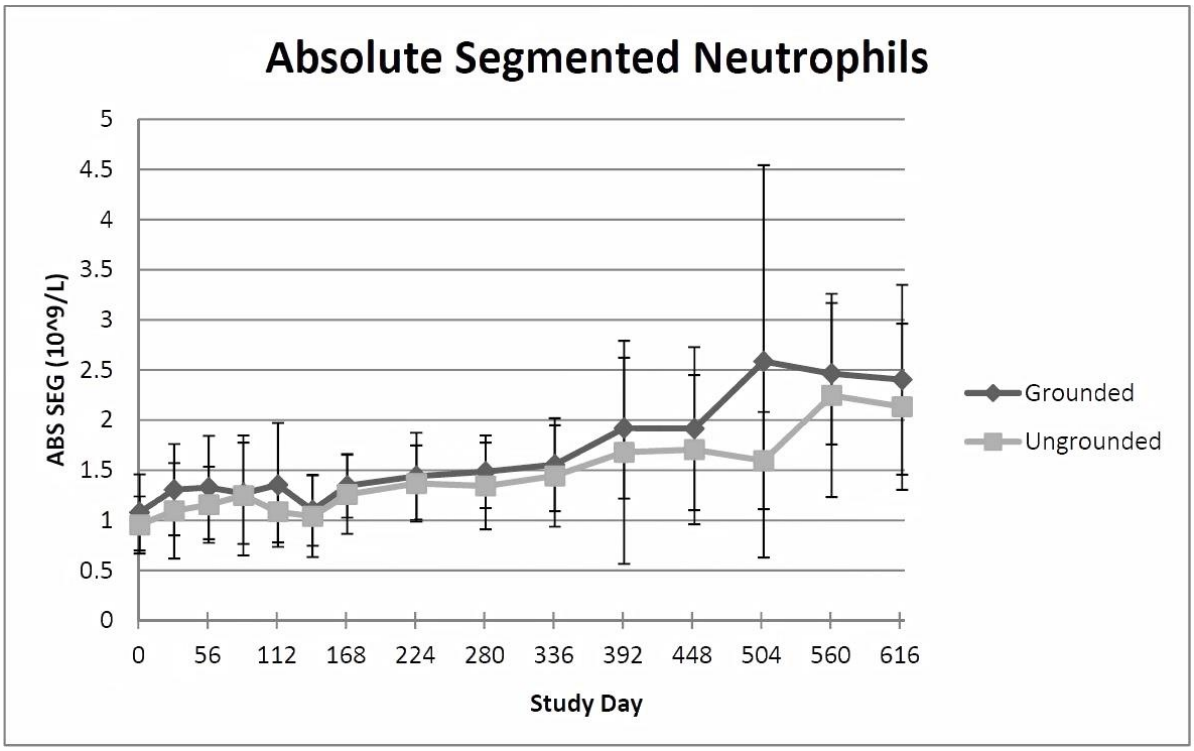

Figure 7. Mean ± SD absolute segmented neutrophil (ABS SEG) counts over the course of the study.

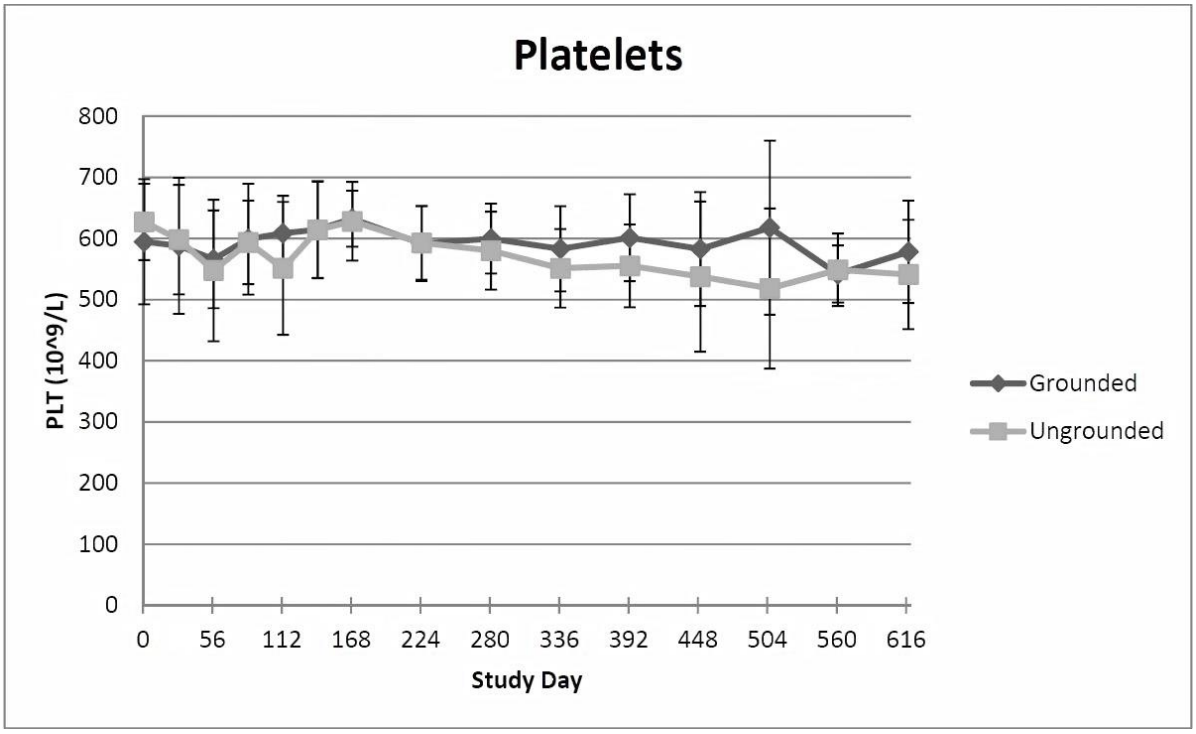

Figure 8. Mean ± SD platelet (PLT) counts over the course of the study.

Interpretation: The differences observed between the groups are small and would normally be interpreted as not clinically significant. Nevertheless, these differences should be noted because they may be part of a treatment-related pattern that is not currently apparent. The ranges fall close to those reported by Charles River Laboratories for their female CD rats of a similar age (see http://www.criver.com/en-US/ProdServ/ByType/ResModOver/ResMod/Pages/CDRat.aspx?TabId=2&SearchTypeId=1&FirstFilter=ShowAll, Biochemistry and Hematology for Lewis Rat Colonies in North America for January 2008 – December 2011). Differences in strain, reference laboratory, location and time are sources of potential differences between the study and reference data, however the reference data do provide a general basis for comparison.

5.5 Clinical Chemistry

AST, ALP (also at Study Day 169), UREA (only at Study Day 169), CRE (also at Study Day 169), GLOB (also at Study Day 169), AG RATIO, GLU (also at Study Day 169), TG (also at Study Day 169), TP (also at Study Day 169), K and CL had significant group differences (Table 9 and 10). Table 9 presents means and standard deviations (SD) for clinical chemistry parameters by group and study day. Table 10 presents results of statistical tests comparing differences between groups while Table 11 presents differences between groups for all clinical chemistry parameters on Study Day 1.

Table 9. Summary Statistics for Clinical Chemistry Parameters by Group and Study Day

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)
CK	Mean	388.87	719.97	494.03	376.13	403.57	345.20	722.33	694.37	363.87	419.37
	SD	344.09	903.06	352.39	396.12	303.59	228.19	706.78	500.42	208.24	188.16
AST	Mean	78.80	93.97	118.90	106.13	97.60	71.70	89.93	112.83	92.73	83.73
	SD	17.34	43.52	58.47	92.34	44.58	16.34	26.30	47.45	66.78	31.72
ALT	Mean	38.67	41.93	55.13	53.93	49.27	32.63	45.73	55.23	50.47	43.70
	SD	13.12	15.29	26.01	33.16	20.06	11.02	9.51	20.77	26.68	9.20
SDH	Mean	20.07	36.37	47.23	37.30	40.87	15.07[1]	38.07	50.00	31.50	33.07
	SD	8.59	17.57	17.71	26.37	16.86	6.20	10.77	20.13	19.74	14.11
ALP	Mean	30.60	46.03	47.50	50.87	45.87	31.17	49.27	56.37	46.63	47.97
	SD	5.56	11.88	7.93	16.27	8.52	6.75	20.53	16.16	8.18	11.17
TBIL	Mean	2.90	2.73	3.00	2.87	2.93	2.87	2.47	2.90	2.83	3.30
	SD	0.61	0.45	0.45	0.43	0.52	0.51	0.51	0.40	0.46	1.34
CA	Mean	2.62	2.54	2.60	2.63	2.63	2.62	2.51	2.56	2.61	2.61
	SD	0.07	0.06	0.05	0.05	0.05	0.06	0.05	0.07	0.06	0.08
PHOS	Mean	1.36	1.15	1.13	1.07	1.08	1.41	1.16	1.09	1.06	1.16
	SD	0.21	0.13	0.12	0.15	0.14	0.12	0.13	0.18	0.17	0.14
NA	Mean	142.63	144.37	144.47	143.97	144.20	142.33	144.53	145.10	143.13	144.17
	SD	1.38	1.00	1.25	1.10	1.27	1.15	1.33	1.42	1.04	1.51
K	Mean	4.03	3.92	3.67	3.54	3.77	4.09	3.94	3.75	3.60	3.86
	SD	0.22	0.25	0.24	0.25	0.22	0.22	0.31	0.21	0.29	0.32
CL	Mean	102.53	100.47	98.73	97.57	95.03	102.50	100.03	98.97	97.17	95.30

Table 9 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)	1 (n=30)	29 (n=30)	57 (n=30)	85 (n=30)	113 (n=30)
CL	SD	1.20	1.48	1.46	1.38	1.19	1.20	1.30	1.19	1.23	1.66
CRE	Mean	34.03	37.47	40.07	35.07	36.10	33.50	36.27	39.20	34.40	34.30
	SD	2.65	4.48	4.40	4.55	3.58	2.26	3.82	4.25	3.83	3.63
UREA	Mean	4.55	6.19	6.48	5.87	5.71	4.25	5.49	6.06	5.46	5.24
	SD	0.49	0.75	0.72	0.76	0.57	0.68	0.78	0.65	0.55	0.78
GLU	Mean	6.64	6.98	6.91	7.07	6.22	6.40	6.85	7.36	7.10	6.58
	SD	0.58	0.74	0.73	0.66	0.70	0.80	0.74	0.78	0.61	0.74
TP	Mean	69.13	71.77	72.90	74.03	74.20	68.57	70.47	71.73	73.10	72.17
	SD	3.74	3.16	2.98	1.85	2.40	3.32	2.96	3.85	3.29	5.27
ALB	Mean	46.27	48.93	50.20	50.13	49.97	45.70	47.97	49.50	49.50	49.23
	SD	3.57	2.52	2.20	1.93	1.67	2.96	2.37	2.91	2.66	3.56
GLOB	Mean	22.87	22.83	22.70	23.90	24.23	22.87	22.50	22.23	23.60	22.93
	SD	1.81	1.58	1.42	1.54	1.76	1.14	1.25	1.52	2.22	2.27
AG RATIO	Mean	2.04	2.15	2.22	2.11	2.07	2.00	2.14	2.23	2.12	2.16
	SD	0.24	0.17	0.14	0.19	0.16	0.15	0.14	0.15	0.23	0.18
CHOL	Mean	3.12	3.45	3.47	3.47	3.61	2.91	3.33	3.44	3.43	3.41
	SD	0.37	0.33	0.27	0.31	0.27	0.34	0.31	0.30	0.33	0.54
TG	Mean	0.76	1.31	1.86	1.74	1.82	0.62	1.04	2.00	1.62	2.14
	SD	0.26	0.48	0.67	0.56	0.61	0.13	0.44	0.69	0.46	0.82

[1]IDNO 45 in Group 2 missing SDH therefore n=29.

Table 9 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	141 (n=30)	169 (n=30)	225 (n=28)	281 (n=28)	337 (n=26)	141 (n=29)	169 (n=29)	225 (n=29)	281 (n=29)	337 (n=27)
CK	Mean	304.27	300.57	531.46	339.54	267.15	245.59	401.86	471.45	421.07	310.78
	SD	227.45	128.95	543.68	205.71	99.02	207.27	241.32	344.60	366.40	293.99
AST	Mean	97.90	90.63	103.00	78.71	77.77	77.66	96.17	91.48	81.31	72.96
	SD	36.45	28.62	38.10	15.53	13.76	24.09	47.35	51.97	29.89	13.39
ALT	Mean	54.87	51.53	56.14	51.68	50.31	51.48	59.03	57.34	55.79	54.59
	SD	15.33	12.37	21.64	8.18	9.54	13.73	23.61	26.02	10.89	11.56
SDH	Mean	27.83	39.17	32.79	25.71	24.04	23.31	40.50[2]	27.55	25.86	23.07
	SD	10.54	13.25	18.68	9.37	5.33	9.74	21.26	13.69	10.65	6.15
ALP	Mean	54.53	50.00	64.61	54.93	65.08	72.93	58.76	67.72	64.00	72.59
	SD	11.00	9.08	17.80	12.56	12.90	27.18	19.04	12.09	13.49	17.21
TBIL	Mean	2.90	2.97	2.96	2.07	2.27	2.76	3.00	2.90	2.31	2.26
	SD	0.40	0.32	0.58	0.38	0.60	0.51	0.60	0.31	0.54	0.59
CA	Mean	2.62	2.61	2.57	2.59[1]	2.63	2.63	2.59[2]	2.56	2.62	2.63
	SD	0.04	0.05	0.04	0.07	0.06	0.07	0.05	0.06	0.07	0.05
PHOS	Mean	0.98	0.99	1.03	1.02	1.07	0.99	1.09	1.05	1.11	1.13
	SD	0.11	0.13	0.12	0.10	0.15	0.21	0.18	0.10	0.13	0.13
NA	Mean	144.17	143.47	143.96	143.32	143.88	144.31	142.55	143.38	143.55	144.22
	SD	1.21	1.33	1.00	1.39	1.18	1.28	1.33	1.01	1.09	0.64
K	Mean	3.71	3.67	3.73	3.82	3.62	3.83	3.84	3.84	3.93	3.67
	SD	0.21	0.21	0.21	0.31	0.22	0.29	0.33	0.36	0.30	0.25
CL	Mean	94.00	101.13	100.25	100.11	98.23	93.90	100.66	100.14	99.07	98.89
	SD	1.89	1.20	1.55	1.81	1.53	1.45	1.78	1.43	2.05	1.67
CRE	Mean	32.60	35.23	35.11	33.93	34.81	30.76	34.66	32.59	33.21	32.85

Table 9 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	141 (n=30)	169 (n=30)	225 (n=28)	281 (n=28)	337 (n=26)	141 (n=29)	169 (n=29)	225 (n=29)	281 (n=29)	337 (n=27)
CRE	SD	4.18	2.92	4.78	3.16	4.95	2.26	3.70	2.10	2.41	3.40
UREA	Mean	5.37	5.41	5.08	4.91	4.55	4.91	5.35	4.94	4.99	4.27
	SD	0.56	0.66	0.85	0.55	0.63	0.59	0.70	0.73	0.47	0.80
GLU	Mean	7.11	6.72	6.80	6.76	6.08	7.78	6.92	6.86	6.69	6.73
	SD	0.88	0.65	0.58	0.81	0.63	0.82	0.62	0.67	0.96	0.71
TP	Mean	72.47	73.63	71.89	71.68	72.92	72.93	71.50[2]	70.21	71.31	70.37
	SD	2.30	2.09	2.85	3.06	3.14	3.81	2.67	2.83	3.36	3.19
ALB	Mean	49.23	50.30	48.57	47.32	47.19	49.55	48.83	48.28	47.90	47.22
	SD	1.59	1.37	1.64	1.85	1.63	3.07	2.55	2.09	2.40	1.80
GLOB	Mean	23.23	23.33	23.32	24.36	25.73	23.38	22.57[2]	21.93	23.41	23.15
	SD	1.63	1.40	2.02	1.68	2.62	1.70	1.71	1.81	2.21	2.61
AG RATIO	Mean	2.13	2.16	2.10	1.95	1.85	2.13[2]	2.18[2]	2.22	2.06	2.07
	SD	0.17	0.13	0.19	0.12	0.18	0.18	0.22	0.20	0.20	0.25
CHOL	Mean	3.38	3.48	3.58	3.64	3.74	3.44	3.44	3.45	3.54	3.63
	SD	0.27	0.24	0.36	0.27	0.29	0.39	0.37	0.39	0.44	0.39
TG	Mean	2.21	2.41	2.17	2.76	2.59	2.57	2.65	2.67	3.44	3.10
	SD	0.51	0.69	0.68	0.82	0.84	0.73	0.80	0.89	1.15	0.92

[1] IDNO 49 missing CA therefore n=27.
[2] IDNO 16 non sufficient quantity (nsq) for SDH, CA, TP, GLOB and A_G therefore n=28.

Table 9 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	393 (n=25)	450 (n=18)	506 (n=10)	561 (n=6)	618 (n=4)	393 (n=26)	450 (n=23)	506 (n=19)	561 (n=12)	618 (n=7)
CK	Mean	531.88	280.89	347.20	365.67	333.50	460.81	304.87	339.97	299.00	275.71
	SD	436.37	124.19	92.93	215.36	51.71	310.62	122.29	223.75	64.55	85.66
AST	Mean	92.16	87.67	93.90	91.50	232.00	78.08	74.83	81.79	87.92	115.29
	SD	30.06	26.40	27.52	13.17	248.16	11.80	12.60	10.82	25.46	67.07
ALT	Mean	46.08	48.78	52.50	50.17	81.00	50.31	48.57	47.95	50.33	51.57
	SD	6.96	17.55	9.35	11.69	99.27	9.36	14.75	8.21	18.93	20.18
SDH	Mean	30.92	28.00	37.20	17.00	37.50	24.35	25.45[1]	27.00	14.33	28.17[2]
	SD	18.20	9.03	10.42	5.02	36.60	10.04	8.52	8.22	6.21	14.70
ALP	Mean	52.24	46.89	54.00	47.83	46.25	60.81	50.96	57.79	59.33	64.14
	SD	11.08	17.94	10.81	7.88	14.71	11.62	21.01	27.01	14.75	27.76
TBIL	Mean	2.60	2.17	3.50	4.17	3.00	2.50	2.35	3.42	3.25	3.14
	SD	1.41	0.38	0.53	0.41	0.82	0.51	0.57	0.51	0.45	0.38
CA	Mean	2.57	2.57	2.57	2.58	2.59	2.59	2.62	2.60	2.62	2.66
	SD	0.05	0.06	0.05	0.05	0.08	0.06	0.06	0.08	0.07	0.09
PHOS	Mean	1.09	1.12	1.13	1.25	1.83	1.14	1.22	1.11	1.27	1.72
	SD	0.17	0.15	0.13	0.11	0.35	0.12	0.10	0.15	0.12	0.21
NA	Mean	142.00	143.22	143.20	140.67	147.75	141.46	142.00	143.32	142.08	147.86
	SD	1.29	1.31	0.79	1.51	1.71	1.70	0.85	1.29	1.62	1.07
K	Mean	3.58	3.55	3.51	3.22	3.45	3.67	3.71	3.66	3.60	3.81
	SD	0.28	0.33	0.26	0.23	0.44	0.29	0.30	0.23	0.24	0.31
CL	Mean	99.40	99.00	94.80	94.00	100.00	99.23	98.39	97.00	97.75	101.71
	SD	1.83	2.11	1.93	1.10	2.16	2.34	2.08	2.05	2.49	1.89
CRE	Mean	33.32	27.94	31.50	30.00	25.25	31.65	27.13	31.84	28.33	26.71
	SD	3.86	4.28	2.51	2.45	4.99	3.20	3.56	3.30	1.97	5.02

Table 9 continued…

		Grounded (Group 1)					Not Grounded (Group 2)				
Parameter	Study Day	393 (n=25)	450 (n=18)	506 (n=10)	561 (n=6)	618 (n=4)	393 (n=26)	450 (n=23)	506 (n=19)	561 (n=12)	618 (n=7)
UREA	Mean	4.21	3.72	3.97	4.00	4.10	4.44	3.84	4.07	4.52	5.16
	SD	0.62	0.60	0.44	0.57	0.82	0.53	0.47	0.80	0.78	0.65
GLU	Mean	5.98	6.07	5.45	5.62	6.30	6.71	6.61	6.31	5.58	7.27
	SD	1.04	1.29	1.44	0.84	1.34	0.86	0.89	1.18	0.87	2.46
TP	Mean	72.16	72.06	72.10	74.33	66.25	70.31	70.87	70.84	71.50	66.00
	SD	4.21	3.47	3.28	2.94	3.30	3.18	3.55	3.76	3.83	3.61
ALB	Mean	45.48	44.17	42.40	43.17	37.50	45.69	45.70	44.63	43.08	39.57
	SD	3.03	2.31	3.78	0.75	3.32	2.02	1.79	1.54	2.47	2.82
GLOB	Mean	26.68	27.89	29.70	31.17	28.75	24.62	25.17	26.21	28.42	26.43
	SD	2.56	3.85	5.29	3.43	5.32	2.37	2.77	3.31	2.75	2.15
AG RATIO	Mean	1.72	1.61	1.48	1.40	1.36	1.87	1.84	1.73	1.53	1.51
	SD	0.18	0.22	0.33	0.17	0.37	0.20	0.20	0.21	0.17	0.15
CHOL	Mean	3.41	3.53	3.72	4.29	3.13	3.43	3.62	3.84	3.98	3.37
	SD	0.46	0.54	0.43	0.50	0.41	0.39	0.42	0.83	0.52	0.64
TG	Mean	2.19	1.49	1.77	1.62	1.29	2.76	2.72	2.35	2.12	1.98
	SD	0.93	0.69	1.06	0.57	0.46	0.74	1.32	0.83	0.84	0.81

[1] IDNO 64 missing SDH therefore n=22.
[2] IDNO 22 missing SDH therefore n=6.

Table 10. Results of Statistical Analyses of Clinical Chemistry Parameters

Parameter	Effect	Significance Level (p-value)	Summary of Significant Group Differences
CK[1]	Group	0.9005	
	Study Day	<0.0001	
	Group by Study Day	0.7244	
	Predose Covariate	0.8312	
AST[1]	Group	0.0086	Group 1 had sig. higher AST than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.1587	
	Predose Covariate	0.0248	
SDH[1]	Group	0.0997	
	Study Day	<0.0001	
	Group by Study Day	0.2714	
	Predose Covariate	0.0227	
ALT[1]	Group	0.6592	
	Study Day	<0.0001	
	Group by Study Day	0.6275	
	Predose Covariate	0.5892	
ALP[1]	Group	0.0004	Group 1 had sig. lower ALP than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0326	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.5390	
TBIL[1]	Group	0.7004	
UREA	Group	0.8968	
	Study Day	<0.0001	
	Group by Study Day	0.0007	Group 1 had sig. higher UREA than Group 2 on sday 29. (p=0.0427).
	Predose Covariate	0.0053	
CRE	Group	0.0127	Group 1 had sig. higher CRE than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.8436	
	Predose Covariate	0.0252	
TP[1]	Group	0.0011	Group 1 had sig. higher TP than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0283	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.0009	
ALB	Group	0.5166	
	Study Day	<0.0001	
	Group by Study Day	0.0026	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.0135	
GLOB[2]	Group	<0.0001	Group 1 had sig. higher GLOB than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0013	Group 1 had sig. higher GLOB than Group 2 on sdays 337 (p=0.0177), 450 (p=0.0442) and 506 (p=0.0018).
	Predose Covariate	0.4583	

[1] No change in conclusions when outliers were removed.
[2] When outliers were removed, overall group difference still present, however group difference only significant for sday 337.

Table 10 continued…

Parameter	Effect	Significance Level (p-value)	Summary of Significant Group Differences
AG RATIO	Group	<0.0001	Group 1 had sig. lower AG Ratio than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0060	Group 1 had sig. lower AG Ratio than Group 2 on sday 337 (p=0.0078), 450 (p=0.0133), and 506 (p=0.0131).
	Predose Covariate	0.3848	
CA	Group	0.4583	
	Study Day	<0.0001	
	Group by Study Day	0.0236	No sig. pairwise group differences on specific study days.
	Predose Covariate	<0.0001	
PHOS[1]	Group	0.1589	
	Study Day	<0.0001	
	Group by Study Day	0.0278	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.3590	
GLU[1]	Group	<0.0001	Group 1 had sig. lower GLU than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0110	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.0953	
CHOL[1]	Group	0.7736	
	Study Day	<0.0001	
	Group by Study Day	0.0157	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.0727	
TG[1]	Group	0.0001	Group 1 had sig. lower TG than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.0008	Group 1 had sig. lower TG than Group 2 on sday 450 (p=0.0002).
	Predose Covariate	0.1792	
NA	Group	0.5130	
	Study Day	<0.0001	
	Group by Study Day	<0.0001	No sig. pairwise group differences on specific study days.
	Predose Covariate	0.0012	
K	Group	0.0003	Group 1 had sig. lower K than Group 2.
	Study Day	<0.0001	
	Group by Study Day	0.7086	
	Predose Covariate	0.0404	
CL	Group	0.0774	
	Study Day	<0.0001	
	Group by Study Day	<0.0001	Group 1 had sig. lower CL than Group 2 on sday 561 (p=0.0083)
	Predose Covariate	0.0275	

[1] No change in conclusions when outliers were removed.

Table 11. Differences between groups for clinical chemistry parameters on Study Day 1

Parameter	Sex	Sample Size	EFFECT	Significance Level for Group (p-value)	Interpretation of Significant Findings‡*
TBIL*	F	60	GROUP	0.8161	
ALP‡	F	60	GROUP	0.8176	
ALT‡	F	59	GROUP	0.0248	GROUNDED Signif. GT NOT-GROUNDED
AST‡	F	60	GROUP	0.0784	
SDH‡	F	59	GROUP	0.0058	GROUNDED Signif. GT NOT-GROUNDED
CK‡	F	60	GROUP	0.7259	
GLU	F	60	GROUP	0.1844	
A_G	F	60	GROUP	0.5035	
GLOB	F	60	GROUP	1.0000	
ALB	F	60	GROUP	0.5061	
TP	F	60	GROUP	0.5371	
TG	F	60	GROUP	0.0103	GROUNDED Signif. GT NOT-GROUNDED
CHOL	F	60	GROUP	0.0267	GROUNDED Signif. GT NOT-GROUNDED
PHOS	F	60	GROUP	0.2186	
CA	F	60	GROUP	0.9000	
CL	F	60	GROUP	0.9144	
K	F	60	GROUP	0.3184	
NA	F	60	GROUP	0.3643	
UREA	F	60	GROUP	0.0559	
CRE	F	60	GROUP	0.4042	

‡ANOVA done on Log transformed values
*The Cochran – Mantel – Haenszel row mean score statistic was used
SDAY – Study Day; GT – Greater Than; Signif – Significantly;

Up to Study Day 169, ALP, CRE, GLOB, GLU, TG, TP and UREA were the only clinical chemistry parameters that showed significant group differences.

- In the grounded group, ALP, GLU, and TG were lower overall than in the ungrounded group.
- CRE, GLOB, TP, UREA were higher in the grounded group than in the ungrounded group.

Considering the entire duration of the study (616 days), ALP (also at Study Day 169), AST, CRE (also at Study Day 169), GLOB (also at Study Day 169), AG Ratio, GLU (also at Study Day 169), TG (also at Study Day 169), TP (also at Study Day 169), K and CL, were the only clinical chemistry parameters that showed significant group differences over the course of the study (UREA was significant only at Study Day 169) and:

- In the grounded group, ALP (also at Study Day 169), AG Ratio, GLU (also at Study Day 169), TG (also at Study Day 169), and K were lower overall than in the ungrounded group.
- AST, CRE (also at Study Day 169), GLOB (also at Study Day 169), and TP (also at Study Day 169) were higher in the grounded group than in the ungrounded group. At Study Day 169, UREA was also higher.

In addition, the following statistically significant observations appeared by Study Day:

- ALP: In the ungrounded group, ALP was greater on Day 141 than on the other days.
- CHOL: In the grounded group, CHOL was less on Day 141 than on Day 113.
- GLOB: In the grounded group, GLOB was less on Day 29 than on Day 113, less on Day 57 than on Day 85 or 113, and in the ungrounded group, less on Day 57 than on Day 85.
- GLU: On Day 141, GLU was less in the grounded group than in the ungrounded group. In the grounded group, GLU on Days 29, 57, 85, and 141 was greater than on Day 113. In the ungrounded group, GLU on Days 29, 85, 113 and 169 was less than on Day 141 and on Day 57 was less than on Day 113.
- NA: In the ungrounded group, NA was higher on Days 29 and 57 than on Days 85 or 169, and was higher on Days 113 and 141 than on Day 169. NA was lower on Day 85 than on Days 113 and 141.
- PHOS: In the grounded group, PHOS was greater on Days 29 and 57 than on Days 141 and 169. In the ungrounded group, PHOS was greater on Day 113 than on Day 85 or Day 141 and HOS was greater on Day 29 than on Day 141.
- TBIL: On Day 29, TBIL was higher in the grounded group than in the ungrounded group. In the ungrounded group, TBIL was less on Day 29 than on Days 1, 57, 85, 113, 141 or 169 but was higher on Day 113 than on Day 141. In the grounded group, TBIL was less on Day 29 than on Days 57 and 169.
- TG: In the grounded group, TG was less on Day 29 than on Days 57, 141 and 169, less on Day 57 and Day 85 than on Day 169, and less on Day 113 than on Days 141 and 169. In the ungrounded group, TG was less on Day 29 than on Days 57, 85, 113, 141, and 169, on Day 57 was less than on Days 141 and 169, on Day 85 was less than on Days 57, 113, 141 and 169, and on Day 113 was less than on Days 141 and 169.
- TP: In the ungrounded group, TP was less on Day 29 than on Day 85.
- AG Ratio: The grounded group had lower AG Ratios than the ungrounded group on Days
    - 337, 450, and 506.
- GLOB: In the grounded group, GLOB was significantly higher than in the ungrounded group
    - on Days 337, 450 and 506.
- TG: In the grounded group, TG was less on Day 450.
- UREA: The grounded group had higher UREA levels than the ungrounded group on Day 29.
- CL: The grounded group had lower CL than the ungrounded group on Day 561.

Plots of ALP (Figure 9), AST (Figure 10), CRE (Figure 11), AG Ratio (Figure 12), CL (Figure 13), GLOB (Figure 14), GLU (Figure 15), K (Figure 16), TG (Figure 17), TP (Figure 18) and UREA (Figure 19) over time are found below.

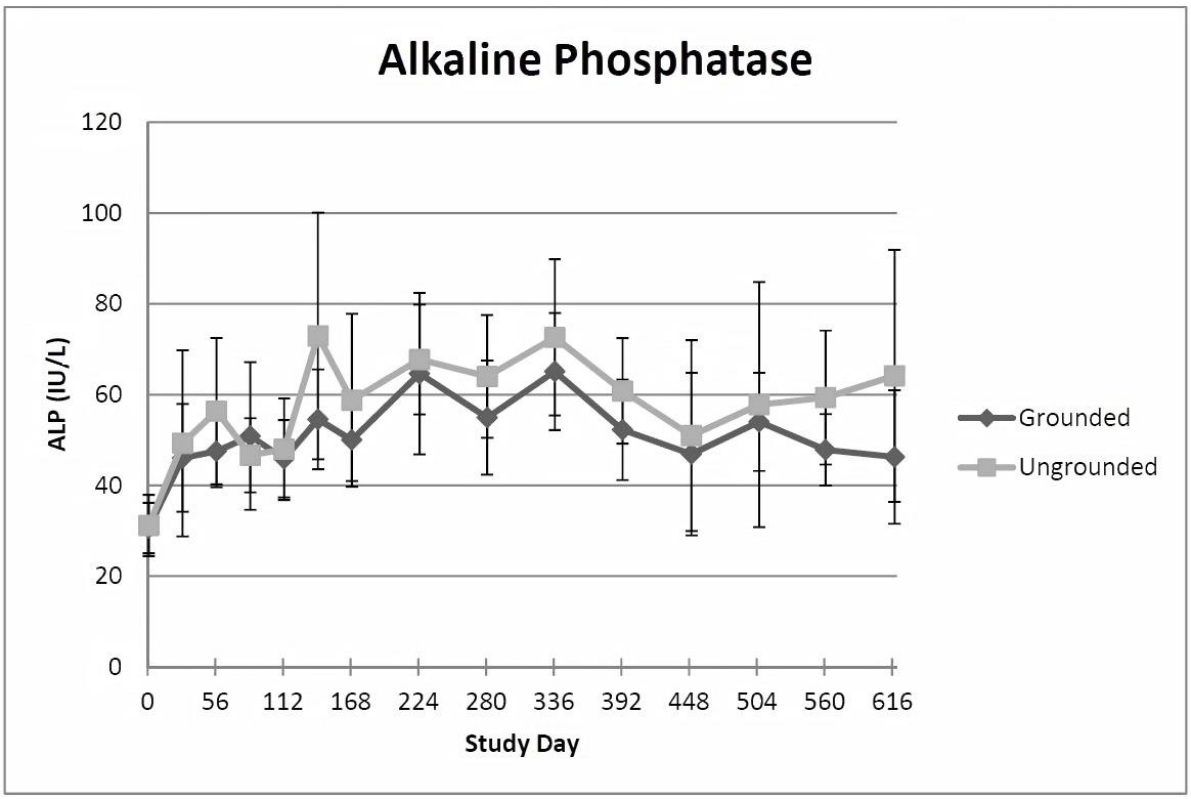

Figure 9. Mean ± SD serum alkaline phosphatase (ALP) concentrations during the study.

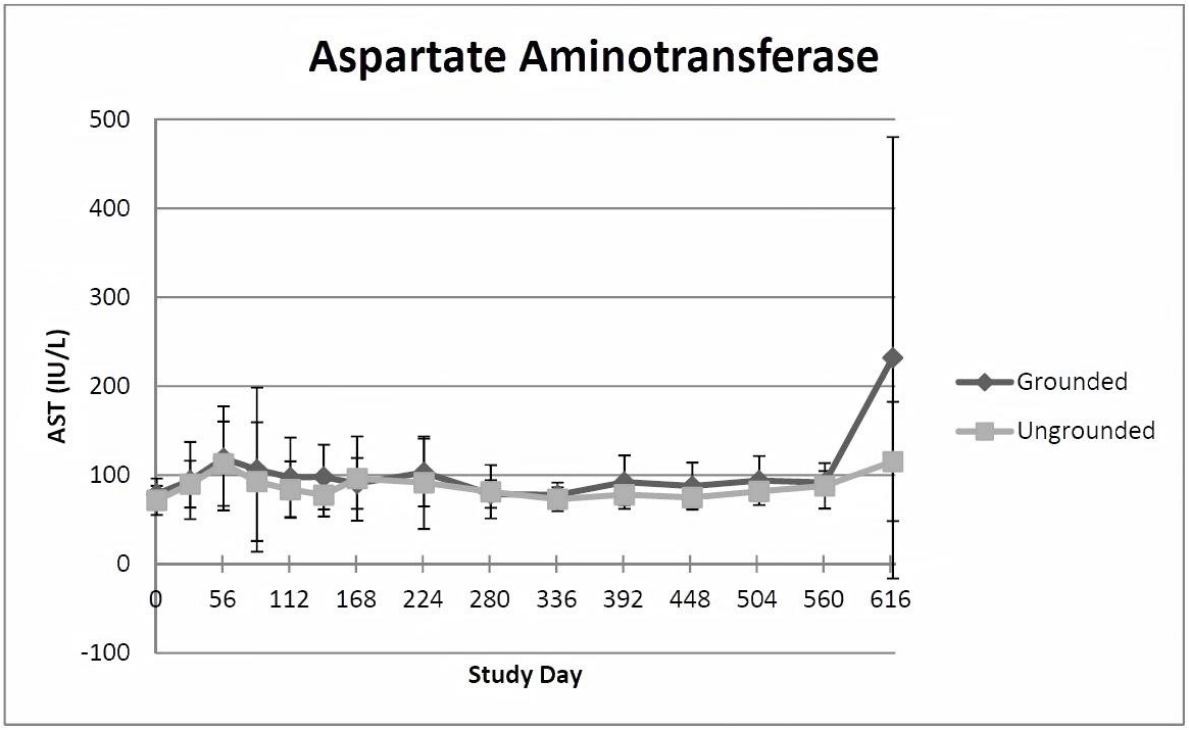

Figure 10. Mean ± SD serum aspartate aminotransferase (AST) concentrations during the study.

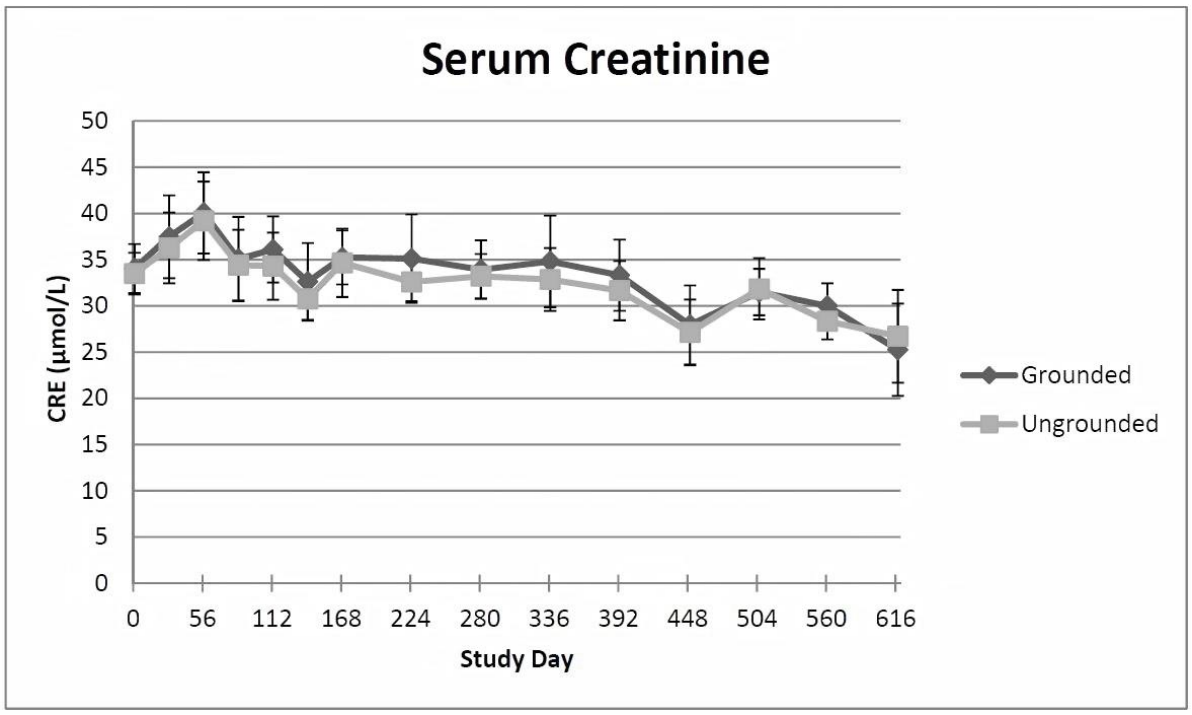

Figure 11. Mean ± SD serum creatinine concentrations over the course of the study.

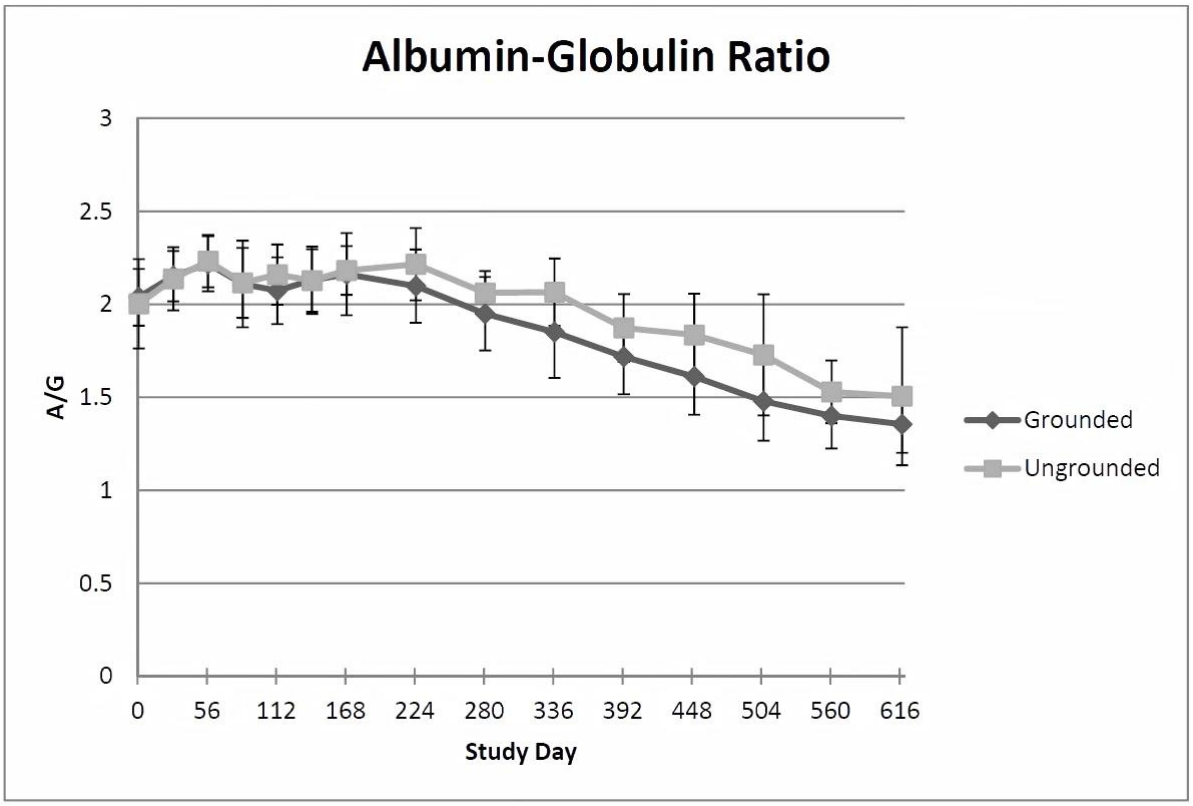

Figure 12. Mean ± SD AG ratios over the course of the study.

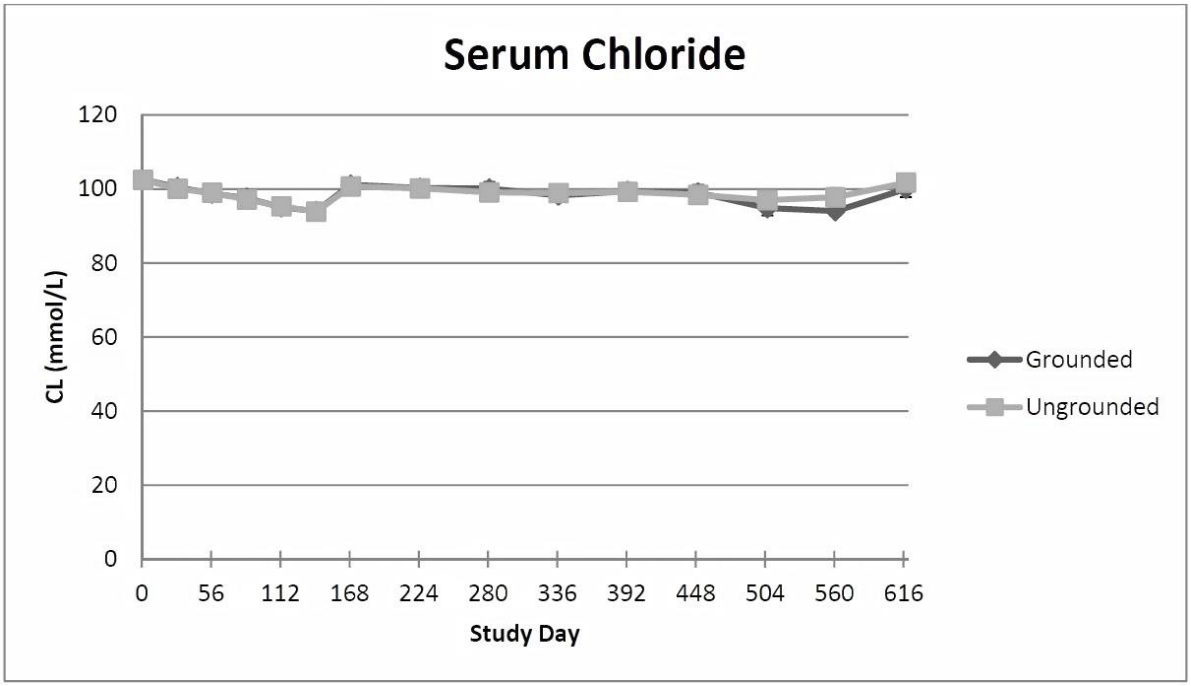

Figure 13. Mean ± SD serum chloride concentrations over the course of the study.

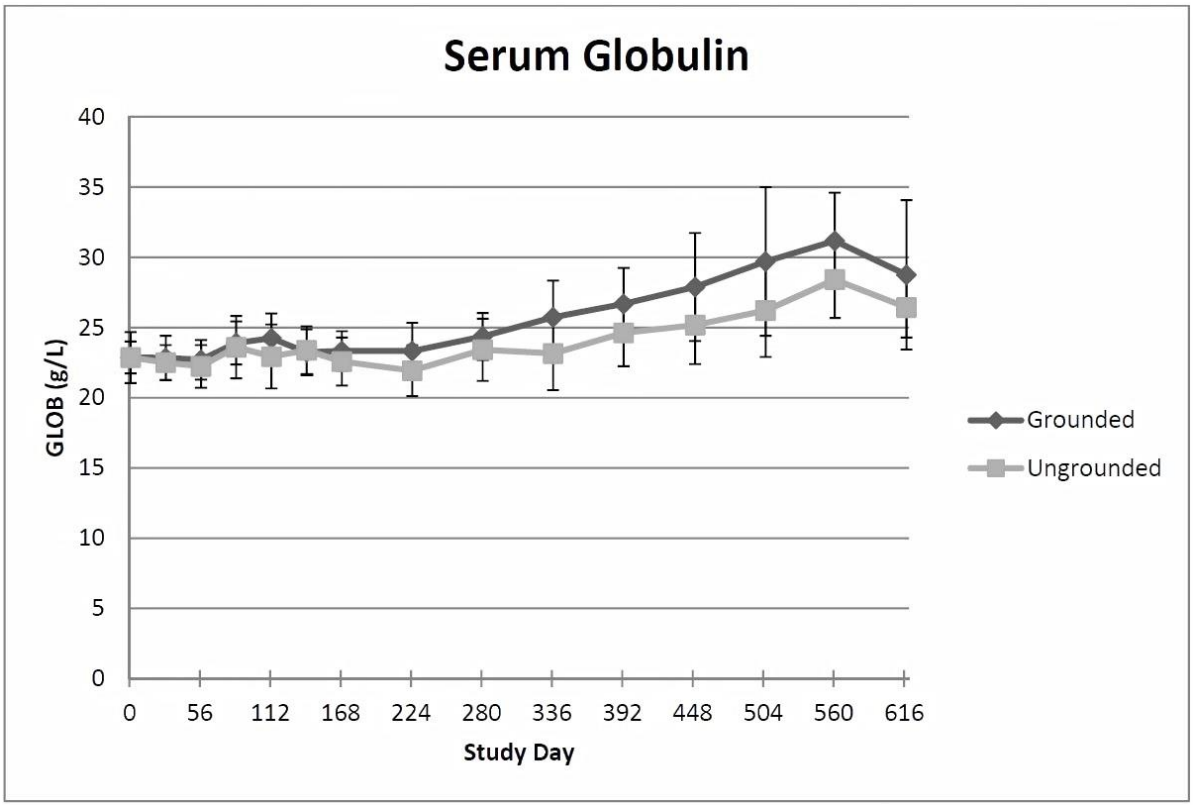

Figure 14. Mean ± SD serum globulin concentrations over the course of the study.

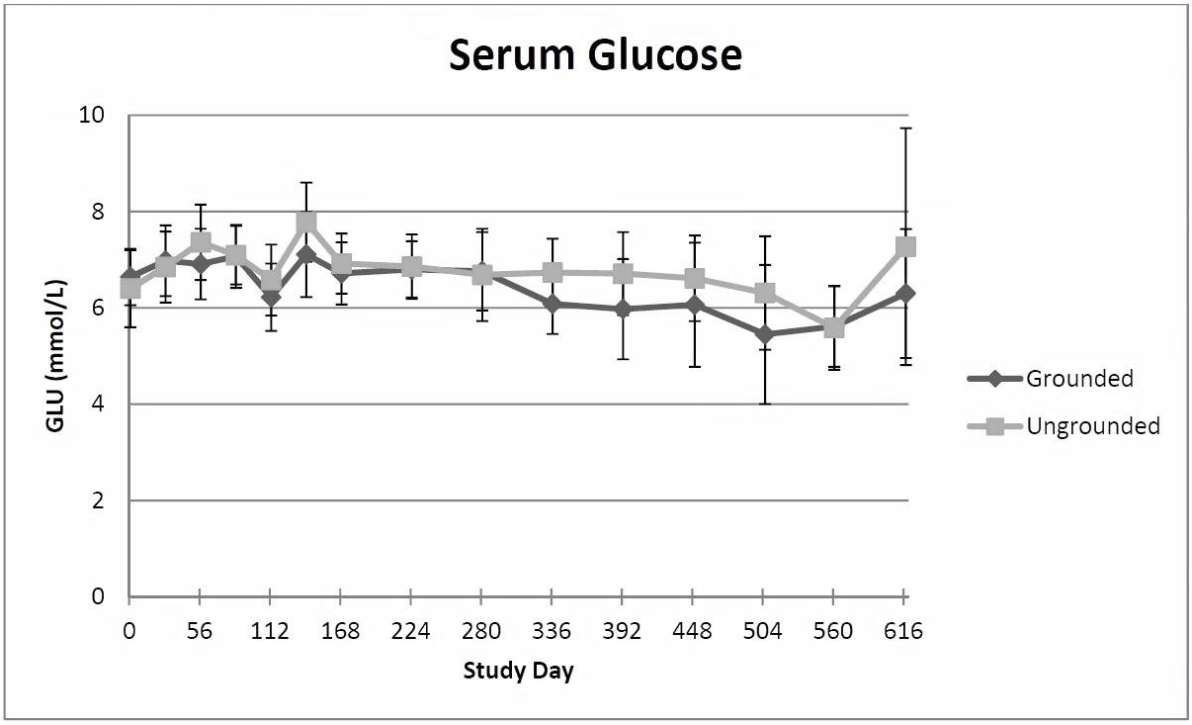

Figure 15. Mean ± SD serum glucose concentrations over the course of the study.

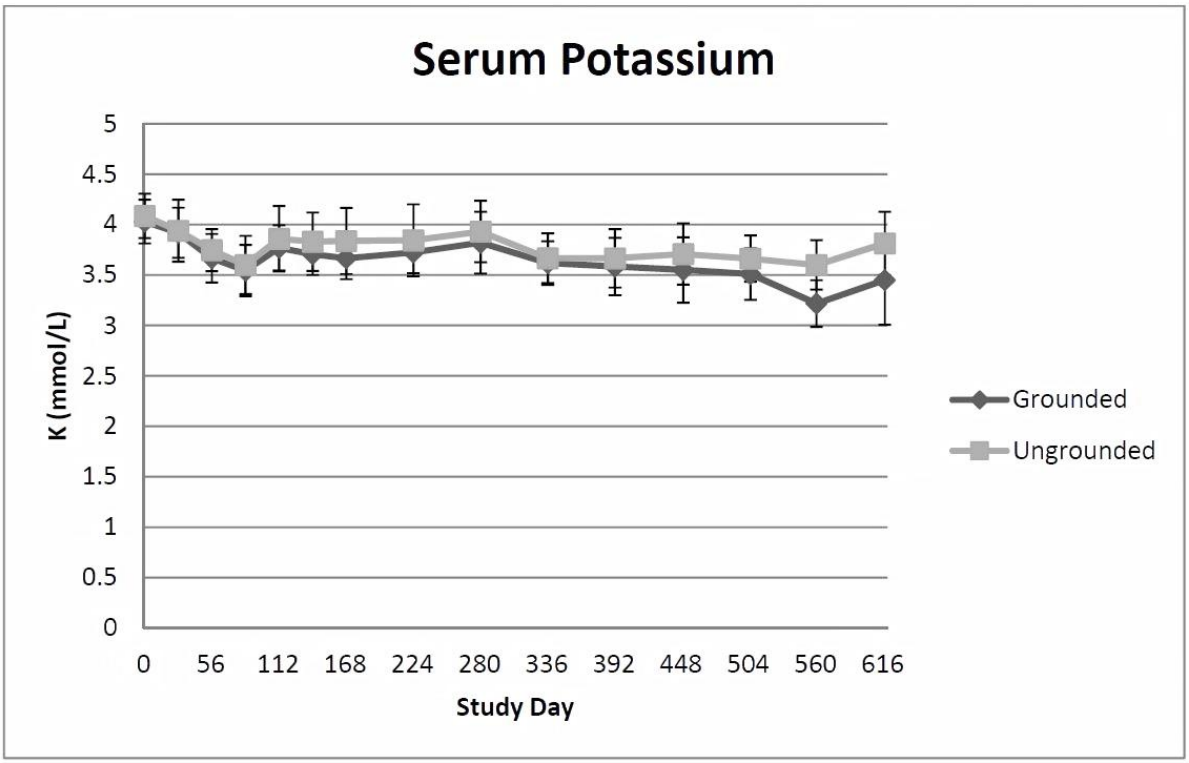

Figure 16. Mean ± SD serum potassium concentrations over the course of the study.

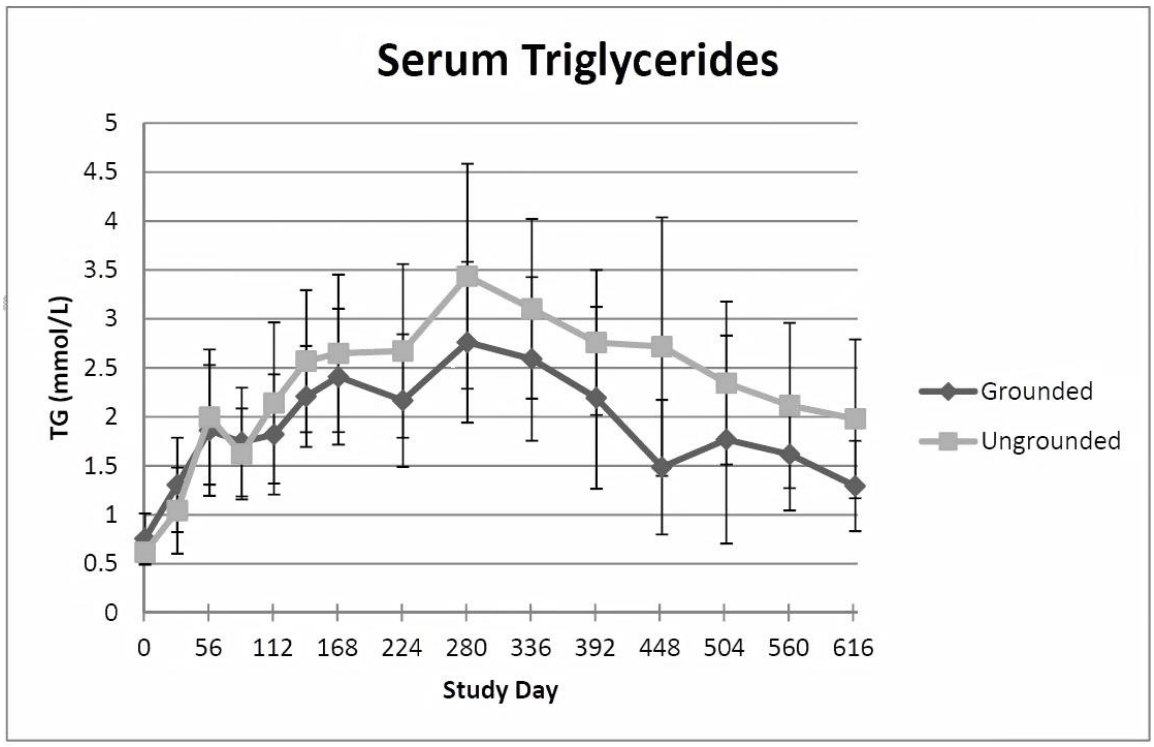

Figure 17. Mean ± SD serum triglyceride concentrations over the course of the study.

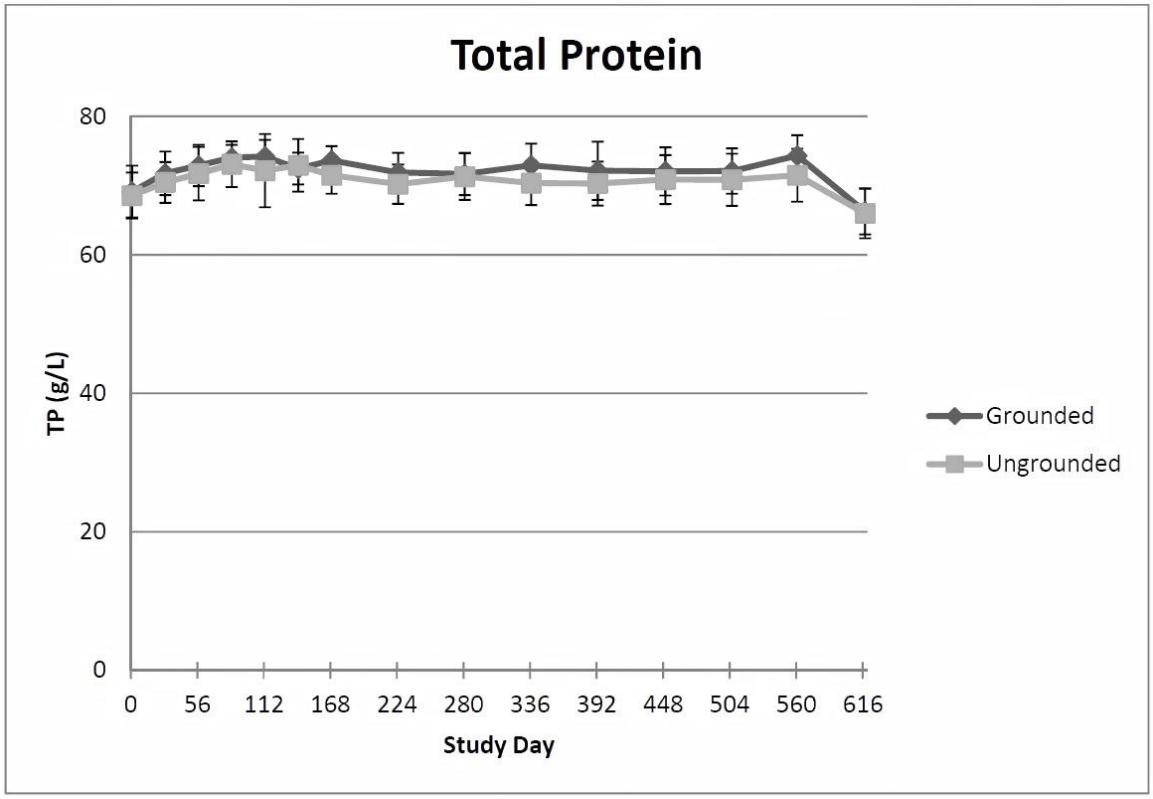

Figure 18. Mean ± SD total protein concentrations over the course of the study.

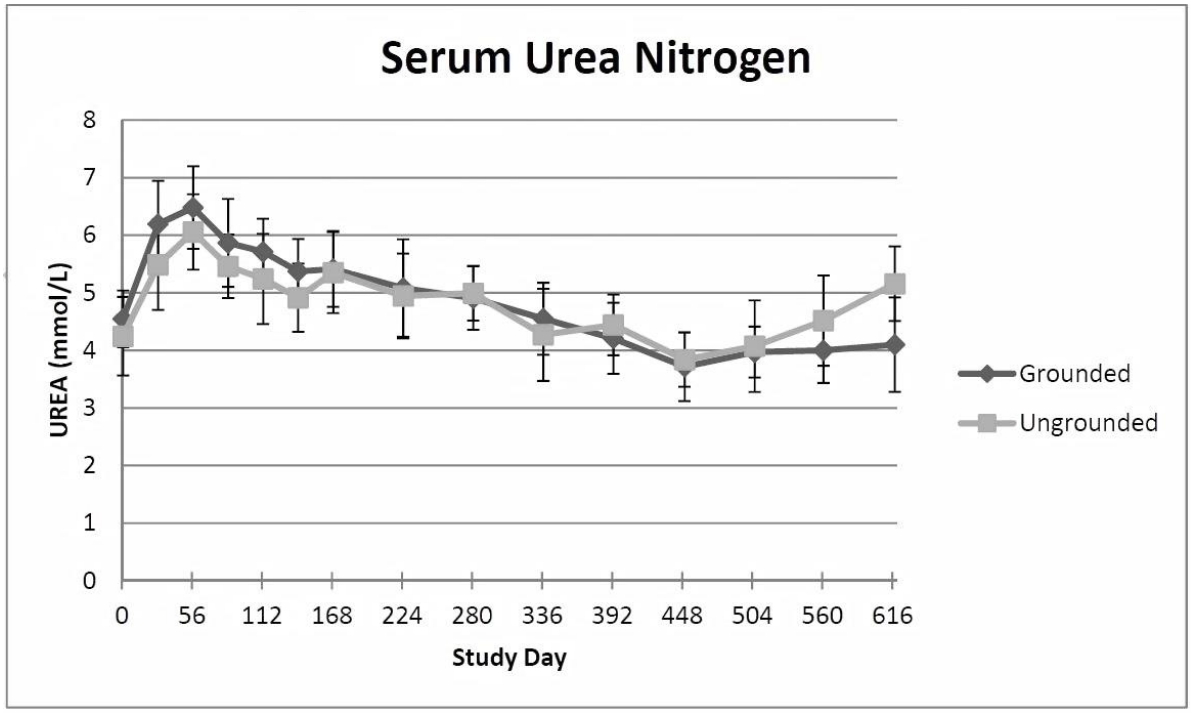

Figure 19. Mean ± SD serum urea nitrogen concentrations over the course of the study.

Interpretation: Similarly to the situation with the hematology data, the differences observed between the groups are small and would normally be interpreted as not clinically significant, especially since they mostly lie within a range considered to be normal (see http://www.criver.com/en-US/ProdServ/ByType/ResModOver/ResMod/Pages/CDRat.aspx?TabId=2&SearchTypeId=1&FirstFilter=ShowAll, Clinical Laboratory Parameters for Crl:CD(SD) Rats - March 2006 Table 22: Summary of Serum Chemistry Historical Control Data from Rats 48 – 65 Weeks of Age – Females, and again, differences in strain, reference laboratory, location and time are sources of potential differences between the study and reference data, so the reference data should be considered to provide only a general basis for comparison). However, data with similar properties are often encountered in large clinical trials when the investigators are considering risk factors rather than outright pathology. Since earthing is hypothesized to cause small physiological changes that over time might affect risk factors for diseases or syndromes, it may be helpful to consider statistically significant differences in related parameters to determine if patterns are discernable that might lend support to the hypothesis.

All of alkaline phosphatase, triglycerides and glucose were lower in the grounded group and this relationship was evident for the duration of the study. All three parameters may be related to the metabolic syndrome, where lower levels are associated in humans with less risk of the diseases associated with the metabolic syndrome such as hypertension, dyslipidemia, obesity and Type 2 diabetes. Rat models of the metabolic syndrome, such as Wistar Ottawa Karlsburg W rat show much larger differences from normals, and these differences progress over time (1).

Creatinine, produced upon breakdown of muscle cells, and filtered by the kidney, was higher in the grounded group than in the ungrounded group, indicating slightly lower kidney function or higher muscle turnover in the grounded group. Whereas the preliminary report at the 169 day

point of the study indicated that UREA also followed this pattern, overall for the entire study, this relationship did not continue to hold excepting for on Study Day 29. The reason for this difference is not known. Creatinine levels in both groups declined over the course of the study, consistent with reduction of muscle mass with aging.

Globulin is one component of total protein, the other being albumin. Since albumin did not differ significantly between the groups, the differences in total protein can be attributed entirely to differences in globulin levels. The most important globulins are the immunoglobulins and since these are found at higher levels in the presence of higher immune system activity, the presence of higher levels of globulin in the grounded than in the ungrounded group contradict the hypothesis that immune system activity is lower in the grounded state. Globulin levels, however, are not specific for immune activity and other explanations for this observation may arise.

Some of the differences among parameters at various sampling times appear to be random, however triglycerides increased then dropped off after study day 280 as the animals aged. Both groups were similarly affected; therefore this is not a treatment-related affect, but rather a natural progression with aging.

Other parameters showing significant differences at the end of the study but not at the 6-month point include the electrolytes K and Cl and the liver enzyme AST. AST levels in the grounded group slightly but significantly exceeded those in the ungrounded group. Although both group means were in the normal range through the study, the grounded group appears to have had slightly higher turnover of liver cells, leading to higher AST levels. For the electrolytes, CL appeared to be affected mainly near the end of the study (specifically on Study Day 561), when group differences in old age disease processes as the group numbers diminished to low levels may have caused the differences observed in CL levels that were not discernable earlier in the study (see Figure 13). On the other hand, K levels (Figure 16) were lower in the grounded group throughout the study, perhaps reflecting grounding effects on internal cell dynamics.

5.6 Biomarkers

The three biomarkers initially measured included Tumor Necrosis Factor-α (TNFα), nitrate/nitrite (NOx) and C-reactive protein (CRP). In the first 169 day stage of the project, there were no significant differences between groups for NOx. The data were also variable and the decision was made not to continue measurement of this parameter. Therefore, NOx results are reported here up to day 169.

TNFα data are available only to Day 85. The company supplying the ELISA kits for this biomarker changed midway through the analysis. The original kit became unavailable and its replacement performed differently. Therefore, later results would not be comparable to those obtained with the original kit. The clinical pathology department worked with the new supplier to resolve the issue; however, the supplier was unable to furnish kits with suitable sensitivity for the analysis. All samples analyzed with the new kit were below the limits of detection. Therefore, the new assay method may have too low sensitivity and cannot properly measure the TNFα in the study samples. A possible reason for the difference from the original assay may be that the original assay may have lacked specificity and was giving a positive response for analytes other than

TNFα. When this report was finalized, the supplier had not resolved this issue.

Table 12 presents means and standard deviations (SD) for all 3 biomarkers at each measurement day up to Day 169 (Day 85 for TNFα). Table 13 presents statistical tests comparing differences between groups up to Day 169 (Day 85 for TNFα) while Table 14 presents differences between groups for all biomarker parameters on Study Day 1. Finally, Table 15 presents a summary of statistics for CRP biomarker by group and study day all the way to the end of the study.

Table 12. Mean and SD of three biomarker parameters per group at each measurement day

		GROUNDED (N=30)							NOT-GROUNDED (N=30)						
Parameter	Stat	1	29	57	85	113	141	169	1	29	57	85	113	141	169
CRP*	Mean	402.87	254.60	307.67	321.77	363.13	421.43	412.83	365.33	296.27	306.57	327.83	422.47	432.38	386.10
	SD	101.92	112.35	76.60	47.37	99.84	33.35	37.43	106.43	86.77	65.56	38.78	62.02	35.34	44.26
NO*	Mean	42.73	45.80	56.17	34.83	42.80	51.57	65.67	35.97	33.76	59.77	30.57	52.28	49.38	64.38
	SD	16.99	26.51	16.02	18.94	12.70	17.45	18.43	11.67	10.52	15.11	14.27	19.59	17.60	23.26
TNF‡	Mean	140.29	78.50	140.53	125.47	.	.	.	111.47	67.70	75.87	154.53	.	.	.
	SD	363.00	157.25	448.73	355.77	.	.	.	180.71	199.72	144.80	317.00	.	.	.

*IDNO 38 NOT-GROUNDED Euthanized before end of test, missing data for SDAY 141, 169
‡IDNO 10, 17 GROUNDED Missing data for SDAY 1; There were issues with kit, so no data after SDAY 85. Summary is for raw values (not log transformed).

Table 13. Differences between groups for biomarker parameters across study days

Parameter	Sex	Sample Size	EFFECT	Significance Level for Group (p-value)	Interpretation of Significant Findings‡*
CRP	F	60	GRP	0.0292	GROUNDED Signif. LT NOT-GROUNDED
			PREDOSE	0.5750	
			SDAY	<0.0001	
			GRP*SDAY	0.0082	SDAY113: GROUNDED Signif. LT NOT-GROUNDED (P=0.0295); GROUNDED: SDAY29 Signif. LT SDAY85, 113, 141, 169 (P=0.0268, <0.0001,<0.0001, <0.0001); GROUNDED: SDAY57 Signif. LT SDAY141, 169 (P<0.0001); GROUNDED: SDAY85 Signif. LT SDAY141, 169 (P=<0.0001, 0.0002); NOT-GROUNDED: SDAY29 Signif. LT SDAY113, 141, 169 (P<0.0001); NOT-GROUNDED: SDAY57 Signif. LT SDAY113, 141, 169 (P<0.0001); NOT-GROUNDED: SDAY85 Signif. LT SDAY113, 141, 169 (P=<0.0001, <0.0001, 0.0084);
NO	F	60	GRP	0.4964	
			PREDOSE	0.6228	
			SDAY	<0.0001	
			GRP*SDAY	0.0366	GROUNDED: SDAY29 Signif. LT SDAY169(P=0.0015); GROUNDED: SDAY57 Signif. GT SDAY85 (P=0.0004); GROUNDED: SDAY85 Signif. LT SDAY141, 169 (P=0.0192, <0.0001) GROUNDED: SDAY113 Signif. LT SDAY169 (P<0.0001); NOT-GROUNDED: SDAY29 Signif. LT SDAY57, 113, 141, 169 (P=<0.0001, 0.0062, 0.0490, <0.0001); NOT-GROUNDED: SDAY57 Signif. GT SDAY85 (P<0.0001); NOT-GROUNDED: SDAY85 Signif. LT SDAY113, 141, 169 (P=0.0003, 0.0043, <0.0001);
TNF	F	58	GRP	0.8202	
			PREDOSE	<0.0001	
			SDAY	<0.0001	
			GRP*SDAY	0.0020	NOT-GROUNDED: SDAY29 Signif. LT SDAY57, 85 (P=0.0152, <0.0001); NOT-GROUNDED: SDAY57 Signif. LT SDAY85 (P=0.0010);

SDAY – Study Day; LT – Less Than; GT – Greater Than; Signif – Significantly;

Table 14. Differences between groups for biomarker parameters on Study Day 1

Parameter	Sex	Sample Size	EFFECT	Significance Level for Group (p-value)	Interpretation of Significant Findings
CRP	F	60	GROUP	0.1683	
NO	F	60	GROUP	0.0774	
TNF‡	F	58	GROUP	0.1928	

‡ Analysis done on Log transformed values.

Table 15. Summary Statistics for CRP Biomarker by Group and Study Day

	Grounded (Group 1)			Not Grounded (Group 2)		
Study Day	N	Mean	SD	N	Mean	SD
1	30	402.87	101.92	30	365.33	106.43
29	30	254.60	112.35	30	296.27	86.77
57	30	307.67	76.60	30	306.57	65.56
85	30	321.77	47.37	30	327.83	38.78
113	30	363.13	99.84	30	422.47	62.02
141	30	421.43	33.35	29	432.38	35.34
169	30	412.83	37.43	29	386.10	44.26
225	28	274.61	110.21	29	258.41	145.34
281	28	244.29	115.48	29	204.10	70.33
337	26	303.15	128.30	27	282.07	132.27
393	25	258.44	71.16	26	211.65	79.21
450	18	336.00	155.26	23	232.30	90.03
506	10	339.20	227.80	18	231.06	109.87
561	6	311.17	94.20	12	230.83	48.09
618	4	155.75	26.40	7	182.86	84.09

As with the clinical chemistry values, up to study day 169 all three biomarkers showed differences among study days, and the trends were for all three parameters to increase over time in both groups.

Neither NOx nor TNFα showed group differences, however CRP was lower in the grounded group than in the ungrounded group overall, and specifically on Day 113.

Although at Study Day 169, CRP was significantly lower in the grounded group than in the ungrounded group, by the end of the study, this relationship was reversed for the study and overall, CRP was significantly higher in the grounded group (see Figure 20 below). The overall trend in both treatment groups was for CRP to decrease over the course of the study from about

400µg/mL at the beginning to around 200µg/mL at the end of the study. There were no significant pairwise group differences on specific study days.

Interpretation: The observation at the 169 day point in the study that CRP was lower in the grounded group was consistent with the clinical chemistry observations on alkaline phosphatase, triglycerides and glucose; however, this relationship did not hold for the duration of the study and was in fact reversed. Lower levels of all four parameters are associated with lower metabolic syndrome-related risk in humans, and the CRP data are not in line with this relationship in the later stages of the study. At the end of the study, however, the intergroup differences disappeared and the CRP levels became almost identical. It is not possible to explain the higher CRP levels late in the study, as, confounding the interpretation, a number of age-related systemic diseases that could have influenced CRP, especially neoplasms, affected individual rats. It is important to note that the decreasing trend of CRP concentrations overall may constitute evidence that inflammation was in general declined over the course of this study as a natural consequence of aging, despite the use of the sensitive Lewis rat strain.

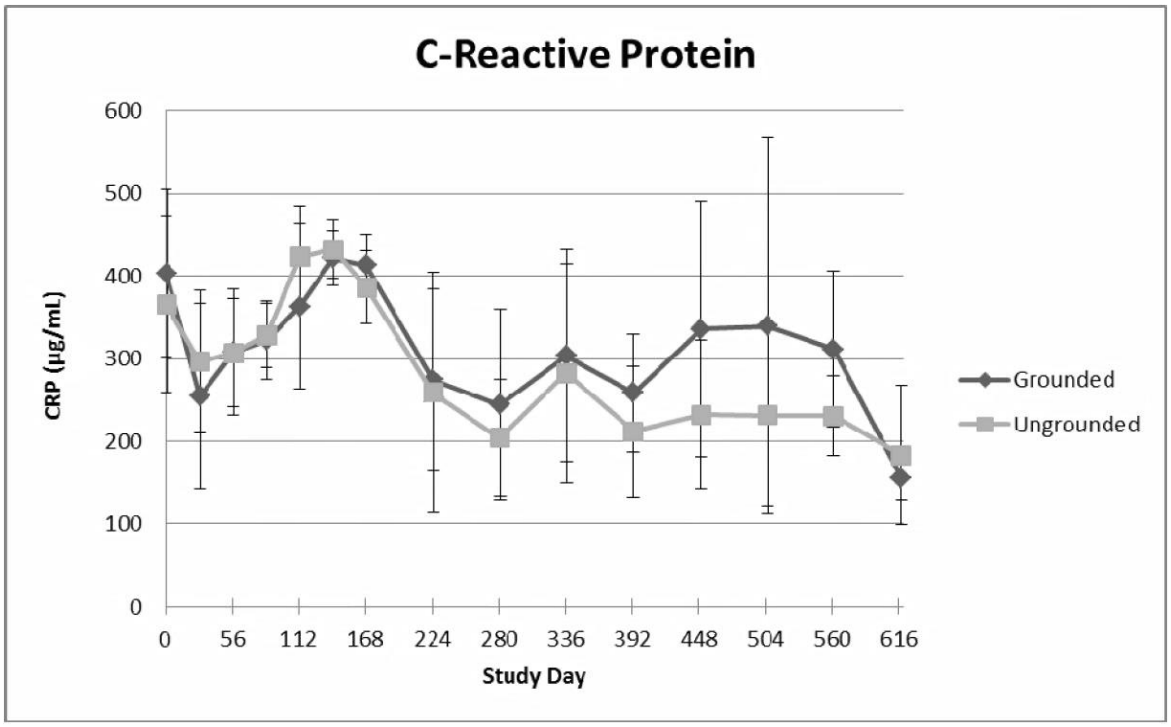

Figure 20. Mean ± SD serum C-reactive protein concentrations over the course of the study.

5.7 Pathological Findings

5.7.1 *Macroscopic Findings*

Raw results for macroscopic finding on necropsy are found in Table 16 below. Given that the study was carried out for the expected lifespan of the rats, especially near the expected end of the natural lives of the animals, a variety of findings was encountered, mostly masses in various locations, especially the mammary glands and pituitary region, but also, less commonly, masses in other locations and skin lesions.

Table 16. Macroscopic Findings

Id. No.	Group	Date	Study Day	Comments
14	1	22-Dec-2008	181	Liver - edges are rounded. Spleen - large clot, enlarged. Intestine - reddened area. Kidney - darkened area in medulla. Uterus - Darkened areas under surface, nothing in lumen. Some digestive material in stomach, blood in chest cavity. Large solid tumor in chest - about 2.5cm in diameter. Appears to have bled out - DIC?
52	1	1-Apr-2009	281	Left and right tarsus - ulceration plantar surface. Left eye opaque.
49	1	30-Apr-2009	310	Inguinal mass about 4cm, firm and freely moveable under skin. Mammary tumor? Left kidney some surface pitting. Uterus- serosal surface slightly reddened and slightly rough. Adrenals- prominent, enlarged?
59	1	8-Jul-2009	379	Lesion from the left perineum area. Uterus- uneven and thick, when palpated can feel lumps
11	1	22-Jul-2009	393	Large mass in left submandibular area, 4cm. Fascia attached, freely moveable under skin. 1-2 mm miliary white masses throughout submandibular area
33	1	27-Jul-2009	397	Dehydrated, stomach empty. Intestine pale. Right kidney slightly mottled. 8mm pituitary mass in floor of cranial vault
12	1	14-Aug-2009	415	Soft 1.5cm peritoneal mass - whitish in color, not very firm. Large dark mass in abdomen - appears at ileocecal junction, lobulated, 4 x 4 cm, blood filled, somewhat necrotic tissue.
24	1	14-Aug-2009	416	Kidney - pits, potential infarction. Good bodily condition. Intestine is full. Mass behind right front limb, caudal, freely moveable under skin, firm and solid; appears glandular, whitish in color. Mammary tumor?
55	1	24-Aug-2009	426	Stomach full. Left hind leg and paw swollen. Swollen muscle appears pale. Fur missing, scabbed around the hock. Loss of blood flow to that region of the body, possible tumor.
44	1	6-Sep-2009	438	Mammary tumor about 1 x 1 cm, white, firm and lobulated. Right and left kidney pitted.
4	1	8-Sep-2009	440	Adrenal left and right - pale in color, white specks. Left slightly enlarged. Pituitary - dark in color, lobulated, vascular, friable, ~ 0.5 x 0.5cm
53	1	17-Sep-2009	450	Kidney's lobulated. A 4 cm mass on the underside of the left hind leg and around the anus. Mass filled with brown-red liquid. Might be necrotic tissue or liquefied tissue. Purulent in areas. Jejunum contained reddish intestinal material - checked for lesions in the GI track, did not find any
20	1	14-Oct-2009	476	Trachea contains food and mucus. 1 cm dark mass on floor of cranial vault beneath the brain, pituitary adenoma? Left kidney pitted. Stomach full, food in mouth. Hole in the right side of upper abdomen, liver damaged. This animal likely aspirated after a seizure, secondary to the mass under the brain
63	1	19-Oct-2009	481	Axilla Lymph Node enlarged. Mass right axilla, pus filled, fibrous. Skin - ulcerative lesions on side and back covering 8 mm in diamete
7	1	21-Oct-2009	484	Thin condition. Pituitary mass 0.8 cm. Brain - left side of cortex is flattened.
65	1	23-Oct-2009	485	Poor bodily condition - body fat depleted; stomach and cecum full; teeth malformed. Left eye protruding, dry and enlarged. Mesenteric lymph nodes slightly enlarged and red. Lymph node, might be salivary? Inflammation observed, left enlarged when compared to right. Left kidney small infarct. Brain - meningeal hemorrhage over cortex, mass along floor of brain (base of skull) left side near pituitary. Retro bulbar mass
37	1	6-Nov-2009	499	Skin erosions throughout. Left and right ears otitis. Left and right eye lids erosions present. Mass subcutaneous inguinal area, contains milk like material, about 2cm in diameter, mammary tumor? Pituitary tumor about 0.5cm in diameter.
46	1	6-Nov-2009	500	Right eye opaque. Right and left kidney pitting throughout and right kidney has some pale areas.

Table 16 continued…

Id. No.	Group	Date	Study Day	Comments
46	1	6-Nov-2009	500	Right eye opaque. Right and left kidney pitting throughout and right kidney has some pale areas.
50	1	6-Nov-2009	500	Small lesions on back. Left eye opaque. Left hind leg - lesions on bottom of foot.
6	1	12-Nov-2009	506	Inguinal tumor about 3.5 x 2 cm, very soft, yellow in color, bilobed; creamy white exudate from one side of mass; other side firm. Mammary tumor? Pituitary mass about 0.8 x 0.8 cm, dark red in color and very soft.
26	1	12-Nov-2009	506	Submandibular mass about 4 x 2.5cm, lobulated, vascularized, bilobed, firm, whitish in color, opaque exudate. Left and right kidneys pitted. Right uterine horn enlarged and firm. Pituitary tumor about 0.3 x 0.3cm, dark red in color and very soft.
1	1	17-Dec-2009	540	Right eye dry and irritated.
15	1	17-Dec-2009	540	Lesion on bottom of right hind foot. Right hind foot mass (1cm in diameter) reddish. Looks like granuloma.
43	1	21-Jan-2010	575	Animal thin. Mass on floor of skull - pale, 8 mm. Left eye opaque, whitish.
40	1	5-Feb-2010	590	Porphyrin staining of nares and hair coat of neck. Several multilobulated masses in left inguinal area, largest is 2 x 2 cm, disc shaped, 2 smaller ones caudal to the main one. Left uterine horn - cyst at base. Cortical pitting in both kidneys - old infarction. Firm, fibrous tumor feel. Pituitary adenoma - 0.5 cm in diameter.
10	1	4-Mar-2010	617	Papilloma on tail, ulceration of skin on right flank. Liver - 3 mm darkened spot right, middle lobe.
17	1	4-Mar-2010	617	Mild alopecia on left side of back. Uterus - small dark brown nodule (2 mm).
8	1	4-Mar-2010	618	Scruffy hair coat, scab on ventral tail at base, exudes green purulent liquid Right mandible - thickened, pale, bony mass; possible callus or tumor. Left flank mass - blood filled cyst; possible hemangioma.
27	1	4-Mar-2010	618	Proliferative plantar lesion on right foot.
38	2	30-Oct-2008	128	Rat's outer appearance was very pale. Pale throughout internal organs, loss of body fat. Mass on left shoulder. Lungs - pale, uneven color throughout. Dark edges, dark 3 mm area under right anterior lobe. Liver - pale areas - up to 2 mm in diameter. Disseminated. Very pale throughout, swollen. Large, focal, pale, firm area (7 mm diameter) on ventral margin of left lateral lobe. Spleen - enlarged.
34	2	1-Apr-2009	281	Large (5 cm) fluid filled cyst at end of uterus. Filled with jelly-like material. Lobulated, disorganized masses inside cyst. Darkened 0.5 cm node in mid-right horn of uterus.
61	2	9-Apr-2009	288	Brain- large (.7cm) mass in floor of cranial vault - pituitary tumor?
31	2	8-Jul-2009	379	Free-moving mass in skin, about 5cm in diameter, tumor 1.5cm thick, whitish, firm but soft, sectioned off, glandular looking. Stomach and cecum both full. Kidney, liver, lungs and heart area all fine.
5	2	10-Aug-2009	412	Subcutaneous mass about 4 x 4 cm in the left inguinal area. Free-moving, firm in some places and soft in others. Mass full of grayish, discolored matter. Glandular in appearance. Possible mammary tumor with necrotic center?
30	2	3-Sep-2009	436	Left adrenal mass about 0.75 cm, enlarged and lobulated.
39	2	11-Sep-2009	443	Liver - very pale. Heart - a bit flaccid. Left ventricle dilated, wall thickness thinner then expected. Intestine and esophagus full of ai

Table 16 continued…

Id. No.	Group	Date	Study Day	Comments
54	2	18-Sep-2009	450	Mass 4 x 4 cm in diameter. Dark in color. Lobulated, located on the end of the right horn of the uterus. Highly vascular, blood filled. Cystic in natur.
47	2	19-Oct-2009	481	Ulcerative lesions on right side and back.
64	2	19-Oct-2009	481	Pituitary mass about 7mm in diameter.
66	2	19-Oct-2009	481	Mass right axilla, lobulated, 1.5cm diameter. Mass right flank, subcutaneous, lobulated and firm. Solid all the way through, 3cm diameter.
13	2	13-Nov-2009	506	Mass left axilla, about 1 x 1cm. Dark red outer shell, creamy white and firm on inside, bilobed. Skull - pituitary enlarged and white. Adrenal glands pale with white specks. Right eye completely opaque. Left eye dark red
57	2	13-Nov-2009	506	Pituitary tumor, about 1.0 x 0.5 cm. Bottom of skull dark red.
2	2	17-Dec-2009	540	Vagina - red mass, does not go beyond the cervix. 1.2cm in diameter.
36	2	17-Dec-2009	540	Hair loss throughout body. Subcutaneous mass left inguinal area. Large whitish nodules. 2.5 x 3 cm.
42	2	17-Dec-2009	541	Severe hair loss and lesions throughout body. Skin - ventral alopecia, miliary red spots, numerous lesions (ulcers). Mass on ventral abdomen, left inguinal area - small whitish nodules.
16	2	30-Dec-2009	554	Hair loss, mature cataract left eye, poor body condition. Mesenteric lymph nodes enlarged, red and uniform. Whole chain appears enlarged and reddened. Pituitary enlarged, very dark red and uniform. About 5 cm in diameter.
18	2	2-Feb-2010	587	2nd digit toe nail on right foot is broken. Small intestine and cecum gas filled.
19	2	2-Feb-2010	588	Patchy hair loss and skin lesions throughout body. Mass behind left axilla 1.5 x 2 cm (Mammary tumor?) Pituitary tumor about 0.5 cm in diameter. Left eye opacity.
23	2	5-Feb-2010	591	Left eye glaucoma, large, firm mass on right axilla. Lymph nodes dark pigmented
41	2	5-Feb-2010	591	Generalized alopecia, scabbing over. Mass at right inguinal area - dark, soft, fleshy, bloody cyst. Mass at left inguinal area - firm, white fibrous mass. Pituitary tumor 0.5 cm lobulated.
62	2	5-Feb-2010	591	General alopecia, scabbing right thorax. Uterus - tumor at base, left horn filled with inspissated blood. Mandibular lymph nodes hyperplastic. Spleen enlarged with adhesions (omentum). Inguinal lymph node is enlarged. Pituitary adenoma, unilateral atrophy of the brain. Should have iron-deficiency anemia - microcytic and hypochromic.
3	2	4-Mar-2010	617	Left horn - brown, firm local swelling.
25	2	4-Mar-2010	617	Alopecia and some scabs on right dorsal thorax. Thymus - right lobe enlarged and dark brown.
32	2	4-Mar-2010	617	General alopecia. Liver - cirrhotic, pale, nodular, small. Mammary mass - fleshy, lobulated, exudes purulent fluid, large bloody cyst caudally.
45	2	4-Mar-2010	617	Right kidney - infarct.
60	2	4-Mar-2010	618	General scruffy, partially alopecic hair coat. Some scabs on lateral thorax. Mammary mass - Globular mass 1.5 cm in diameter, full of purulent material.
51	2	17-Dec-2009	541	Hair loss throughout body. Subcutaneous mass, 3 x 3 cm, left inguinal area - large whitish nodules, pus filled.

5.7.2 ***Histopathological Findings and Diagnoses***

Details of the histopathological findings can be provided upon request. The incidences of findings were compared statistically between groups. In the statistical analysis, it was found that the ungrounded group had a significantly higher diagnosed with renal cortical infarcts than the grounded group. The groups did not differ significantly in terms of the proportion of any of the other diagnoses (Table 17).

All of the findings with respect to the bone structure were reported to involve degenerative changes rather than inflammation.

Interpretation: The pathologist noted that the duration of the study has resulted in the observation of large numbers of lesions that are associated with aging in rats. There was no grounding-associated pattern of lesions, although the grounded group had less alopecia and more renal cortical infarcts. The clinical significance of these is not known, given the lack of other associated pathologies. In short, grounding did not seem to greatly affect the type or frequency of age-associated lesions in Lewis rats. In short, grounding did not seem to greatly affect the type or frequency of age-associated lesions in Lewis rats.

Table 17. Summary Statistics for Diagnoses by Group

Diagnosis		Group 1 (n=30)	Group 2 (n=30)
ALOPECIA local or general	Not Present	28 (93%)	21 (70%)
	Present	2 (7%)	9 (30%)
RENAL CORTICAL INFARCTS	Not Present	22 (73%)	29 (97%)
	Present	8 (27%)	1 (3%)
ADRENAL CYSTS	Not Present	29 (97%)	30 (100%)
	Present	1 (3%)	0 (0%)
MAMMARY ADENOMA	Not Present	20 (67%)	18 (60%)
	Present	10 (33%)	12 (40%)
PITUITARY ADENOMA	Not Present	19 (63%)	21 (70%)
	Present	11 (37%)	9 (30%)
ULCERATIVE DERMATITIS	Not Present	24 (80%)	26 (87%)
	Present	6 (20%)	4 (13%)
HEPATIC ADENOMA	Not Present	29 (97%)	30 (100%)
	Present	1 (3%)	0 (0%)
OTHER TUMOR	Not Present	24 (80%)	24 (80%)
	Present	6 (20%)	6 (20%)
OTHER	Not Present	20 (67%)	21 (70%)
	Present	10 (33%)	9 (30%)

CONCLUSION

At day 169, significant differences between grounded and ungrounded animals were seen in several parameters. Some could be factors forming a pattern that may be related to metabolic syndrome (TG, GLU, ALP, CRP), others to kidney function (CRE, UREA) and still others to factors affecting serum proteins (TP, GLOB). Some parameters showed progressive changes over time (TG, CRP, NOx and TNFα).

Over the entire duration of the study, although the group differences were small, two potentially important patterns were observed. One may be related to a beneficial effect of grounding that could be related to a benefit in metabolic syndrome (i.e. the lower levels of TG, GLU, ALP, CRP at Study Day 169 and body weight in the grounded group). The other may relate to immune responsiveness, as evidenced by the slightly higher levels of GLOB and the related parameter TP as well as lower AG Ratio in the grounded group.

The clinical relevance of other intergroup differences is unclear, such as the grounded group's slight differences in blood cell counts and erythrocyte parameters, the grounded group's higher CRE levels, lower K levels in the grounded group that may be associated with intracellular effects of grounding, the higher AST levels in the grounded group, indicating more hepatocyte turnover, all of which occurred within the reported normal ranges for these parameters and none of which seemed to affect survival rates. Further study of the effects of grounding under different experimental conditions may help elucidate the relevance of our observations.

REFERENCES

1. J van den Brandt, P Kovacs and I Klöting, Metabolic syndrome and aging in Wistar Ottawa Karlsburg W rats. International Journal of Obesity (2002) 26, 573 – 576.