

A Toxicity Study Of *Sutherlandia* Leaf Powder (*Sutherlandia microphylla*) Consumption



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Sponsors:

- 1. Medical Research Council of South Africa**
- 2. National Research Foundation**

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1. Main objective

The purpose of this study was to investigate the possible toxicity of consumption of *Sutherlandia* leaf powder (*Sutherlandia microphylla*) in vervet monkeys (*Chlorocebus aethiops*), by determining a variety of biochemical, haematological, physiological and physical variables. These variables reflect liver, kidney, muscle, respiratory, intestinal, bone and general biological function.

2. Ethics

The study was approved by the Ethics Committee for Research on Animals (ECRA) of the Medical Research Council (Project No. 110).

3. Abbreviations

AST	Aspartate Transferase
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
Ca	Calcium
CK	Creatinine Kinase
Cl	Chloride
LDH	Lactate Dehydrogenase
GGT	Gamma Glutamyl Transferase
Hb	Haemoglobin
Hct	Haematocrit
HDL-C	High Density Lipoprotein Cholesterol
K	Potassium
LDL-C	Low Density Lipoprotein Cholesterol
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
Mg	Magnesium
Na	Sodium
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
WBC	White Blood Cells

4. Materials and methods

Note: The project protocol was submitted to the sponsors prior to the start of the study, including detailed proposals of all tests to be conducted, and was agreed upon by all parties. The study was designed to evaluate toxicity but not efficacy.

4.1. Nonhuman primates, environment and housing

All vervet monkeys (*Chlorocebus aethiops*) used in the study were maintained in the Primate Unit of the Diabetes Research Group of the MRC under identical housing conditions. The closed indoor environment was maintained at 25 – 27 °C, a humidity of 45%, about 15 air changes/hour and a photoperiod of 12h. All individuals selected for this project were healthy adult males, which were identified with numbers in ink tattoo. Additionally, all cages were marked according to individual, group designation and experiment number. The project was identified and recorded as Experiment 110. The same diet (see 4.3.) was fed throughout the study and water was available *ad lib* via an automatic watering device. All individuals were housed singly during the study but had regular access to exercise cages and environmental enrichment.

4.2. Treatments

4.2.1. Plant material

The plant material used in this study was harvested in the vicinity of Murraysburg, Western Cape Province, South Africa. The plants were identified as *Sutherlandia frutescens*, subspecies *microphylla*, by Professor Ben-Erik van Wyk, Head of the Botany Department of the Rand Afrikaans University (Voucher specimens were dried and stored). Plants were dried at ambient temperature in the shade and leaves and small twigs were utilized. The dried material was powdered using a coffee bean grinder (Robert Bosch Hausgeraete GmbH, E – Nr. MKM 5000) and sterilized with gamma rays at 18.0 kGy (HEPRO, Montague Gardens, Cape Town). Sterilized material was tested for microbial contamination, and no bacterial growth (including *Salmonella* and *Clostridium*), yeasts or moulds were found after 11 hours of incubation (SWIFT Micro Laboratories (Pty) Ltd., Rosebank, Cape Town).

4.2.2. Dose

Adult male vervet monkeys (n=16) were randomly divided into four groups of four individuals each and allocated according to Table 1. The recommended daily dose of *Sutherlandia* leaf powder for humans is 9.0mg/kg bodyweight. This recommended dose is based on the use of two *Sutherlandia* tablets per day, each containing 300mg *Sutherlandia* dried leaf powder.

Table 1. Treatments

Group	<i>Sutherlandia</i> leaf powder concentrations
1	9.0 mg /kg bodyweight (recommended dose)
2	27.0 mg/kg bodyweight (3x recommended dose)
3	81.0 mg/kg bodyweight (9x recommended dose)
4	Control (maize meal)

4.2.3. Duration of treatments

The treatment period was three months.

4.3. Administration of *Sutherlandia* leaf powder

The plant material was mixed into the standard diet, which consisted of 120g of stiff maize porridge, presented as “patties”, containing micro- and macronutrient supplementation. The controls received 120g of standard diet only.

Every individual received an identified amount of food containing the exact dose of *Sutherlandia* leaf powder. Compliance was monitored daily and food consumption/wastage was measured every two weeks.

4.4. Dosing schedule

The diet containing *Sutherlandia* leaf powder was fed every day in the morning throughout the study and the same feeding times were adhered to.

4.5. Clinical monitoring

Blood samples were collected once a month throughout the study. Biochemical variables were determined with a Technicon autoanalyzer and haematological variables with a Coulter STAK S at an accredited laboratory (Pathcare). At the time of blood sampling, body weight, body temperature, respiratory rate, as well as pulse

rate was recorded, and blood pressures were measured using a Dinamap XL vital signs monitor with a neonatal blood pressure cuff #4. For the blood sampling, the vervet monkeys were sedated with Ketamine at 10mg/kg bodyweight intramuscularly and blood was obtained by femoral venipuncture. Urine was collected every two weeks by means of a funnel placed under each cage, and analyzed with urine test strips (UriCheck, RapiMED Diagnostics, South Africa) for leucocytes, nitrite, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin and glucose.

The following tests were conducted on the blood samples (for definition of abbreviations see section 3):

Haematology: RBC, Hb, MCHC, MCH, MCV, RDW, Hct, platelets, Hb, WBC, basophils, eosinophils, neutrophils, monocytes, lymphocytes.

Clinical Biochemistry: bilirubin (total and unconjugated), AST, ALT, ALP, GGT, LDH, CK, protein, albumin, globulin, cholesterol (total, LDL-C, HDL-C) urea, creatinine, Na, K, Cl, Ca, Mg.

4.6. Observations

All individuals were observed daily and any changes in behaviour (e.g. alert, depressed, fearful, unresponsive, confused, excited, irritable, aggressive) were noted. Apart from clinical signs such as loss of appetite and diarrhea, the following criteria were used to determine wellbeing: coat condition, posture, locomotion, activity, vocalisation and activity in the exercise cage.

4.7. Statistics

All variables were analysed by the MRC Biostatistics Unit utilizing the SAS Version 8 statistical package with the Repeated Measures Analysis of Variance; $P < 0.05$ was considered significant. Statistics were generated for time interactions, time-group interactions and differences between each treatment group and the control group.

5. Results

5.1. Preliminary considerations

- Results and observations are reported and interpreted mainly in terms of possible treatment effects and not biological variation.
- The results in a study such as this do not preclude individual susceptibility and response to the consumption of herbal medicines or any other medicinal compound.
- *Sutherlandia* leaf powder was not tested in pregnant and young animals and the results cannot be extrapolated to these groups.

5.2. Compliance

All individuals consumed their food containing *Sutherlandia* leaf powder bush immediately and no habituation was needed. Food consumption was 100% throughout the study period.

5.3. General observations

The *Sutherlandia* leaf powder had no side effects. There were no signs of discomfort, ill health or abnormal behaviour throughout the study.

5.4. Haematology

The treatment was not associated with statistically or clinically significant changes except for the following:

- A statistically significant difference in red blood cells between group 2 and the controls
- A statistically significant difference in the haemoglobin between group 2 and the controls
- A statistically significant difference in the platelets between Group 1 and the controls

None of the changes was clinically significant and the explanations for this conclusion are provided in Table 2, page 7.

5.5. Biochemistry

The treatment was not associated with statistically or clinically significant changes except for the following:

- Statistically significant differences in the globulins between groups 1, 2 and the controls
- A statistically significant difference in CK between group 1 and the controls
- A statistically significant difference in the ALT of group 2 and the controls
- Statistically significant differences in the ALP between groups 1, 2 and 3 and the controls
- A statistically significant difference in the unconjugated bilirubin between group 1 and the controls
- Statistically significant differences in the urea between groups 1, 2 and 3 and the controls
- Statistically significant differences in the total cholesterol in groups 1, 2 and 3 and the controls.
- Statistically significant difference in the LDL cholesterol between group 2 and the controls

None of the changes was considered clinically significant and the explanations for this conclusion are provided in Table 3, pages 9 and 10.

Table 2: Summary of haematological observations

Variable	<i>P</i> < 0.05	Interpretation	Conclusion
Red Blood Cells	Yes Control vs. Gr. 2	No time-group interaction (<i>P</i> =0.1986), no change in group receiving highest dose (see figure 1)	Not clinically significant
Haematocrit	No		
Haemoglobin	Yes Control vs. Gr. 2	No time-group interaction (<i>P</i> =0.2151), fluctuation in control group (see Figure 3)	Not clinically significant
Table 2 continued	<i>P</i> < 0.05	Interpretation	Conclusion
MCV	No		
MCH	No		

MCHC	No		
RDW	No		
White blood cells	No		
Neutrophils	No		
Eosinophils	No		
Basophils	No		
Lymphocytes	No		
Monocytes	No		
Platelets	Yes Control vs. Gr. 1	Change in control group (see Figure 14), no changes in other groups on higher doses	Not clinically significant

5.6. Physiological variables

The treatment was not associated with statistically and clinically significant changes except for the following:

- A significant difference in the mean arterial pressure between group 1 and the controls
- An increase in the heart rate in groups 2 and 3.

None of these changes was clinically significant and the explanations for this conclusion are provided in Table 4, page 10.

Note regarding the heart rate: In view of the increase in heart rates in groups 2 and 3, and despite the lack of statistical significance, an extra measurement was obtained for this variable while the monkeys were still being treated (treatment only stopped when all last results were obtained and verified). This extra measurement clearly indicated a decline and return to baseline values in both groups (see [Figure 42](#)).

Physiological variables can fluctuate due to exogenous factors.

5.7. Urine analysis

No changes consistent with a treatment effect could be observed in any group.

Table 3: Summary of biochemical observations

Variable	<i>P</i> < 0.05	Interpretation	Conclusion
Ca	No		
Mg	No		
Na	No		
K	No		
Cl	No		
Albumin	No		
Globulin	Yes Control vs. Gr.1 Control vs. Gr.2	Decline in both groups, no change in group receiving highest dose (see Figure 21)	Not clinically significant
Total Protein	No		
CK	Yes Control vs. Gr. 1	Considerable fluctuations in the controls and group 1 (see Figure 23), no changes in both other groups receiving higher dose	Not clinically significant
LDH	No		
GGT	No		
ALT	Yes Control vs. Gr. 2	Group 2 peaks sharply in week 8 only (see Figure 26), no changes in other groups	Not clinically significant
AST	No		
ALP	Yes Control vs. Gr. 1, 2 and 3	Change (decline) in control group (see Figure 28)	Not clinically significant
Total Bilirubin	No		
Table 3 continued	<i>P</i> < 0.05	Interpretation	Conclusion
Unconjugated Bilirubin	Yes Control vs. Gr. 1	Fluctuation in control group – no changes in both other groups receiving a higher dose (see Figure 30)	Not clinically significant

Glucose	No		
Urea	Yes Control vs. Gr. 1, 2 and 3	Considerable fluctuation in control group (see Figure 32)	Not clinically significant
Creatinine	No		
Total Cholesterol	Yes Control vs. Gr. 1, 2 and 3	Fluctuation in control group (see Figure 34)	Not clinically significant
HDL	No		
LDL	Yes Control vs. Gr. 2	Fluctuation in control group (see Figure 36)	Not clinically significant

Table 4: Summary physiological observations

Variable	P < 0.05	Interpretation	Conclusion
Body weight	No		
Body temperature	No		
Systolic Pressure	No		
Diastolic Pressure	No		
Mean Arterial Pressure (MAP)	Yes Control vs. Gr. 1	A steep nadir in group 1 at week 8, recovers at week 12 (see Figure 41)	Not clinically significant
Heart Rate	No	No statistical difference but both groups receiving a high dose increased, a fifth measurement indicates a return to baseline values (see Figure 42)	Not clinically significant

P – values of all variables indicating significant differences are provided in table 5.

Table 5: *P* – values of variables with statistically significant differences/changes

Variable	Time interaction	Time - group interaction	Control versus
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Red Blood Cells	0.0541	0.1986	Group 2	0.0410
Haemoglobin	0.1213	0.2151	Group 2	0.0409
Platelets	0.0075	0.2474	Group 1	0.0332
Globulin	0.0013	0.0059	Group 1	0.0011
			Group 2	0.0032
CK	0.8997	0.2333	Group 1	0.0348
ALT	0.0094	0.0355	Group 2	0.0167
ALP	<0.00001	0.0141	Group 1	0.0492
			Group 2	0.0013
			Group 3	0.0103
Unconjugated Bilirubin	0.0739	0.1642	Group 1	0.0165
Urea	0.0787	0.0441	Group 1	0.0145
			Group 2	0.0121
			Group 3	0.0301
Total cholesterol	<0.0001	0.0088	Group 1	0.0356
			Group 2	0.0240
			Group 3	0.0473
LDL cholesterol	0.0020	0.0870	Group 2	0.0315
MAP	0.1413	0.0821	Group 1	0.0423

Note: The statistically significant differences recorded above were all due to fluctuations in the control group or not associated with changes in groups receiving higher doses, as outlined in tables 2,3 and 4, and were not considered clinically significant.

6. Conclusions

Note: These conclusions refer to *Sutherlandia* leaf powder consumption in adult male vervet monkeys for three months.

- At the recommended dose, *Sutherlandia* leaf powder consumption was not associated with toxic or other side effects within the parameters of this study.

Statistically significant differences between this group and the controls were either due to fluctuations in the control group or not associated with changes in both groups receiving a higher dose (see below). The changes were therefore not considered clinically significant.

- At 3x the recommended dose, *Sutherlandia* leaf powder consumption was not associated with any toxic or other side effects within the parameters of this study. Statistically significant differences between this group and the controls were due to fluctuations in the control group or not associated with changes in the group receiving the higher dose (9x). The changes were therefore not considered clinically significant.
- At 9x the recommended dose, *Sutherlandia* leaf powder consumption was not associated with toxic or other side effects within the parameters of this study. Statistically significant differences between this group and the controls were entirely due to fluctuations in the control group and therefore not considered clinically significant.

Unit abbreviations for Appendices I – III

fl	femtolitre
g/dl	gram/decilitre
g/kg bw	gram/kilogram bodyweight
g/l	gram/litre
iu/l	international units/litre
kg	kilogram
mm/Hg	millimetre Mercury

mmol/l	millimol/litre
pg	picogram
rdw	red cell distribution width
umol/l	micromol/litre

APPENDIX I: Haematology

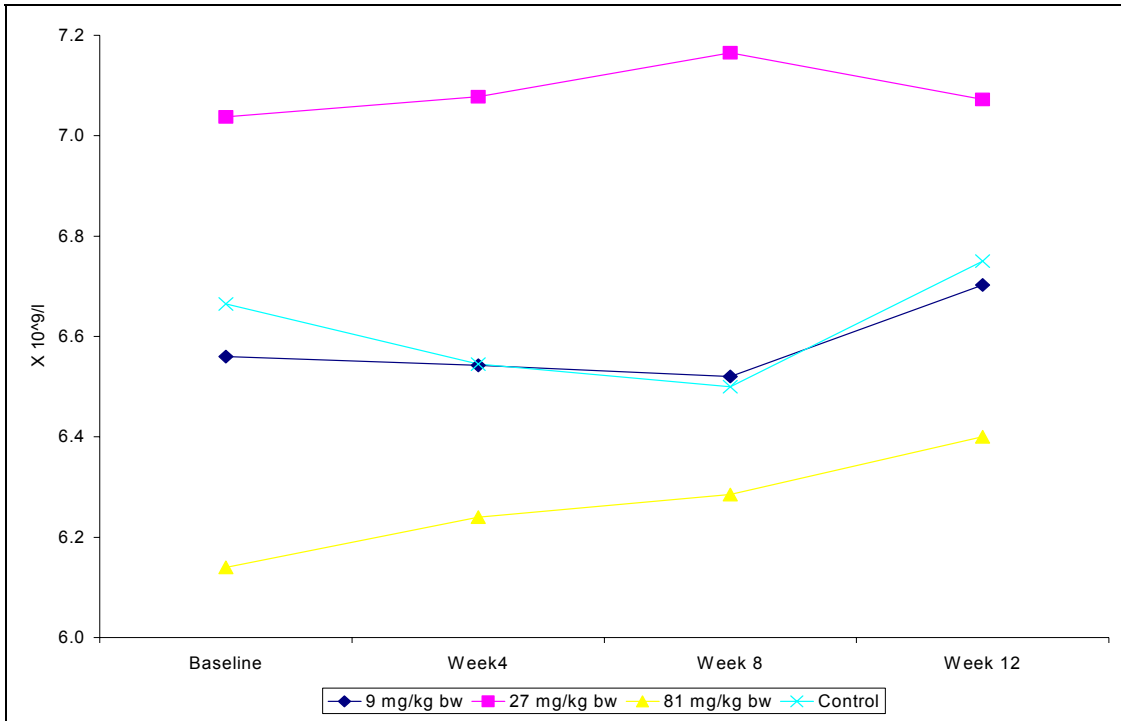


Figure 1. Red blood cells

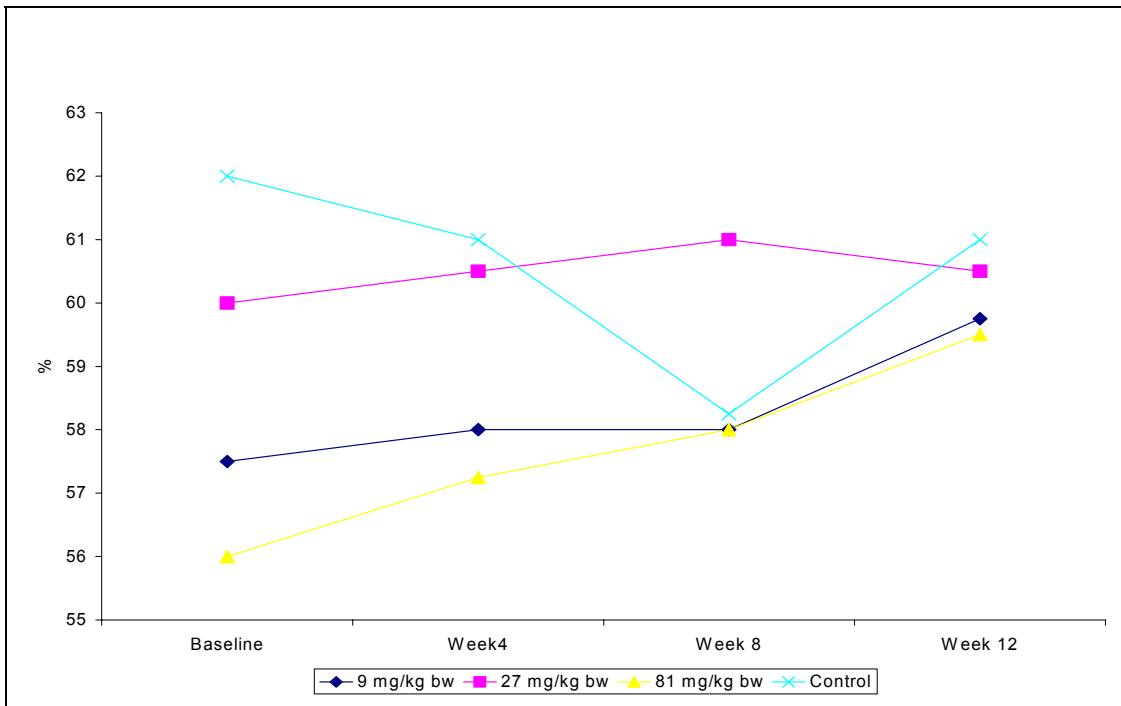


Figure 2. Haematocrit

APPENDIX I: Haematology, continued

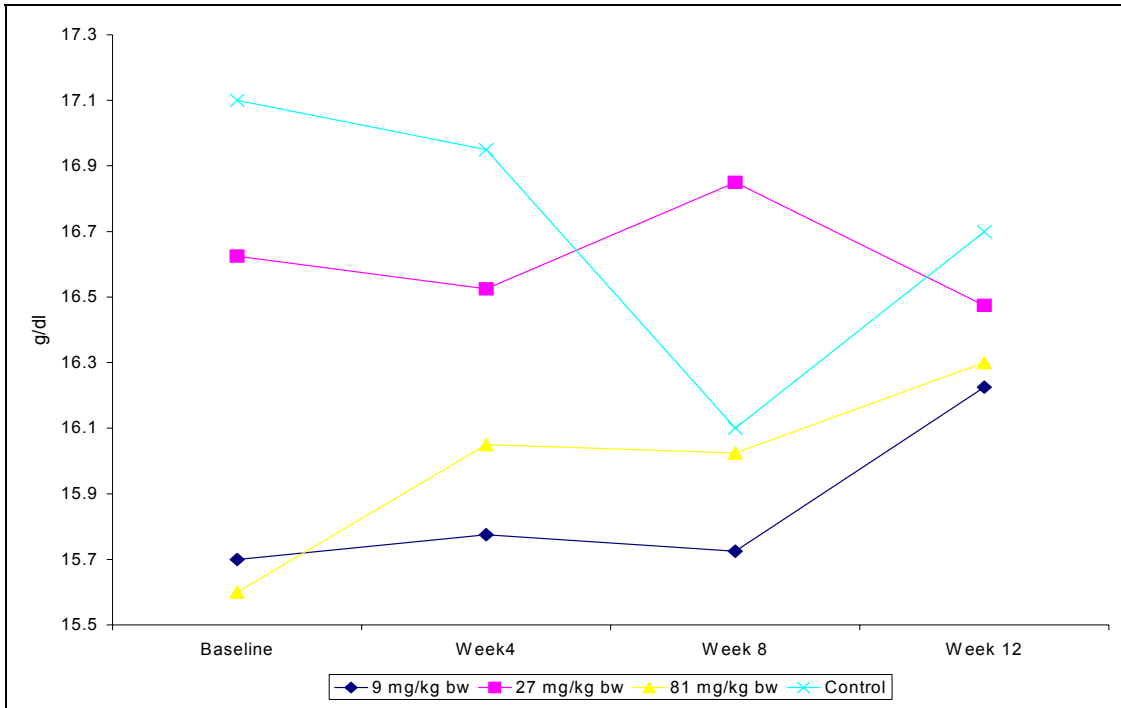


Figure 3. Haemoglobin

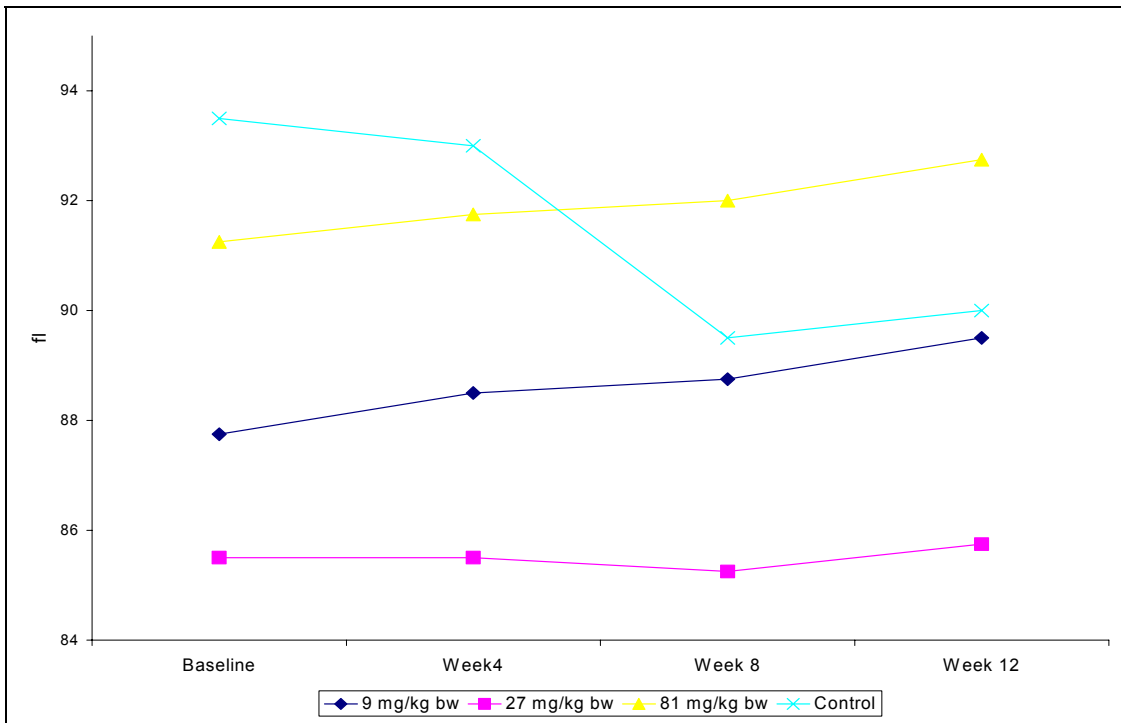


Figure 4. Mean corpuscular volume

APPENDIX I: Haematology, continued

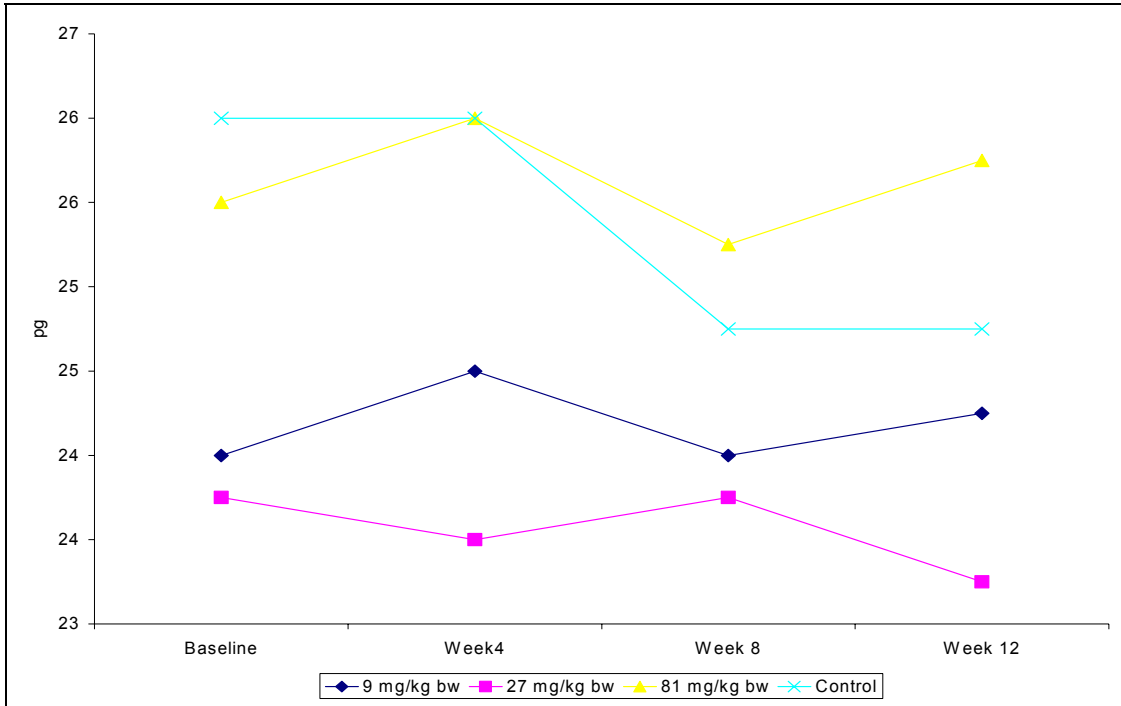


Figure 5. Mean corpuscular haemoglobin

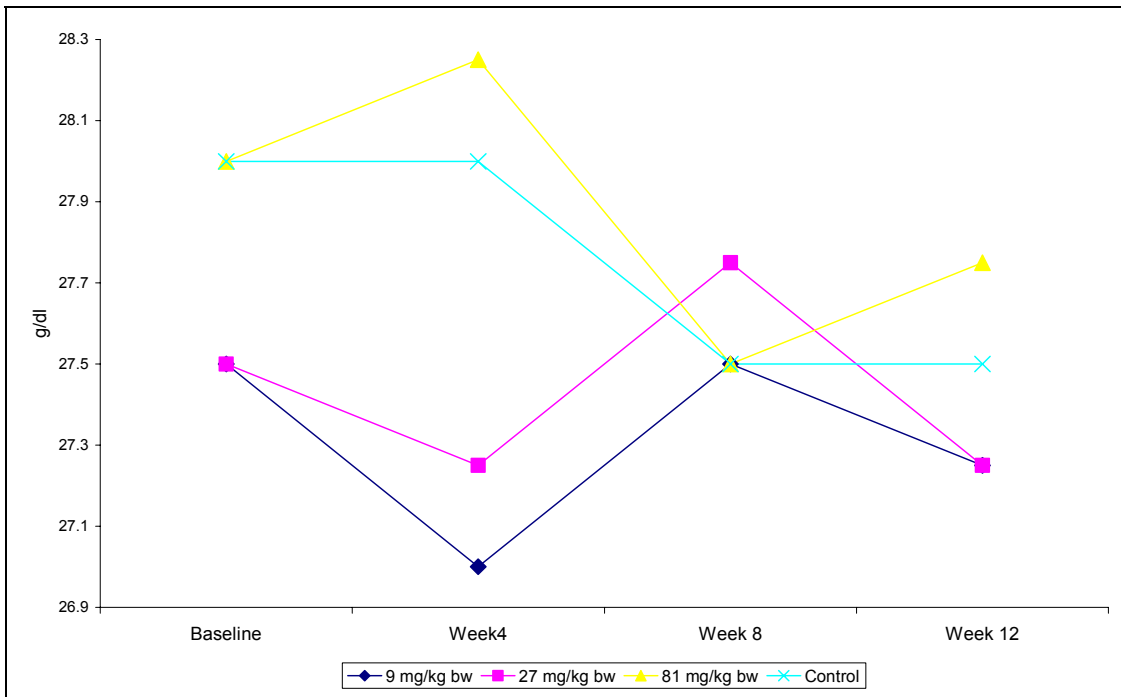


Figure 6. Mean corpuscular haemoglobin concentration
APPENDIX I: Haematology, continued

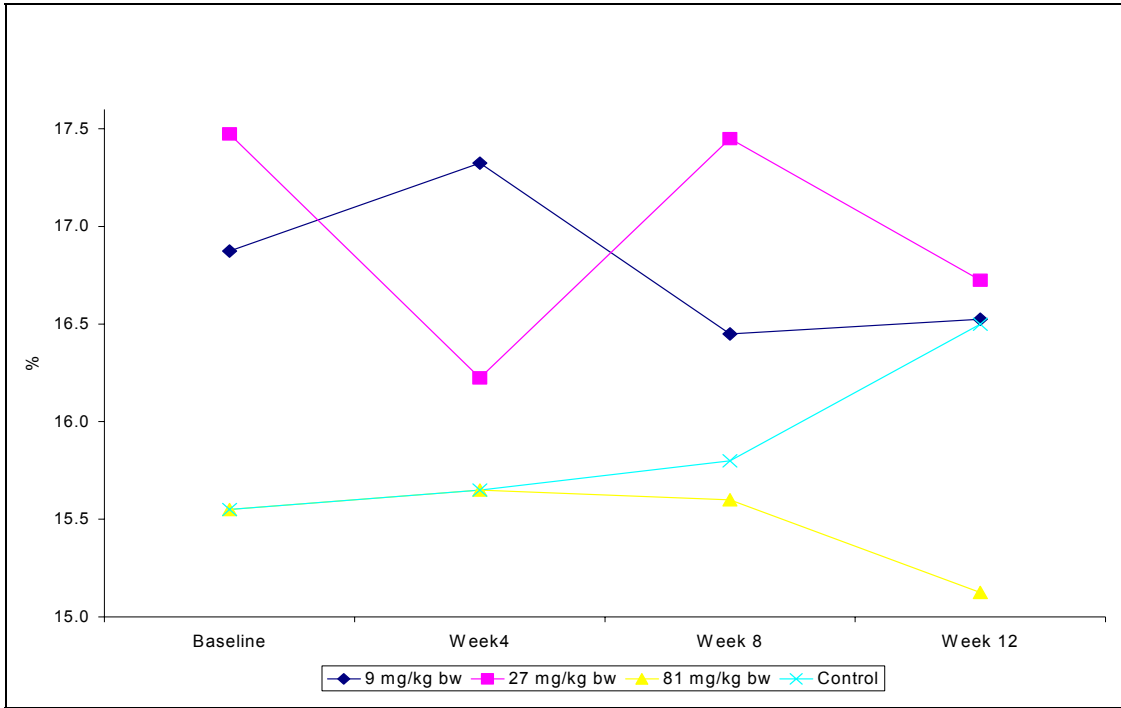


Figure 7. Red cell distribution width

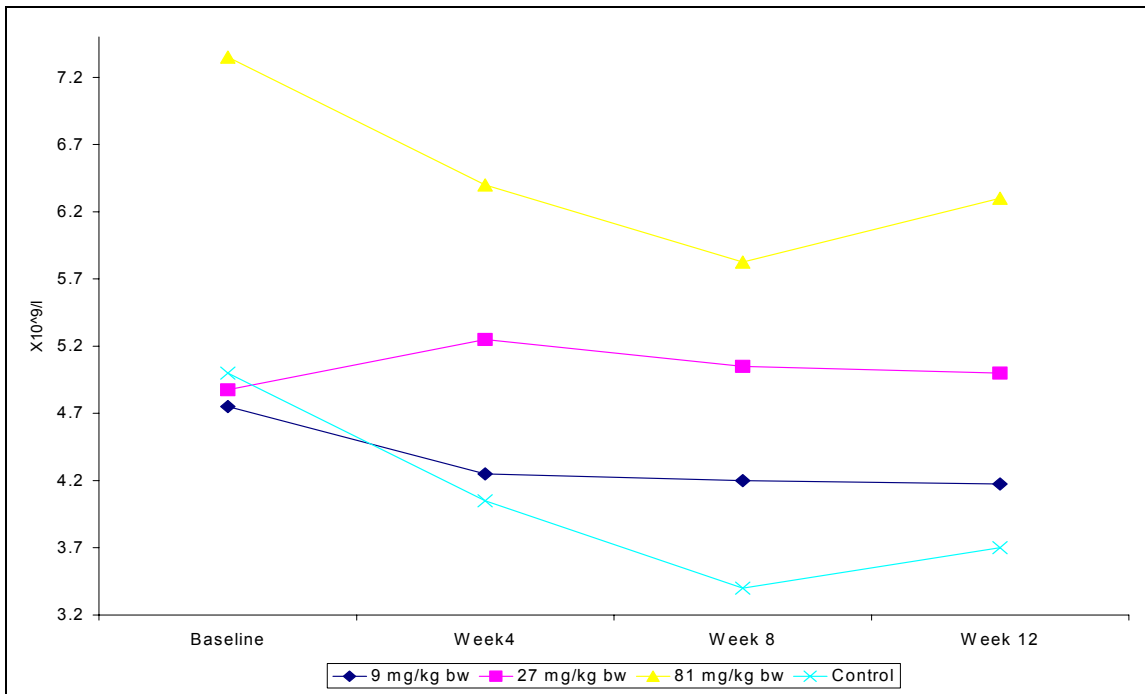


Figure 8. Total white blood cells
APPENDIX I: Haematology, continued

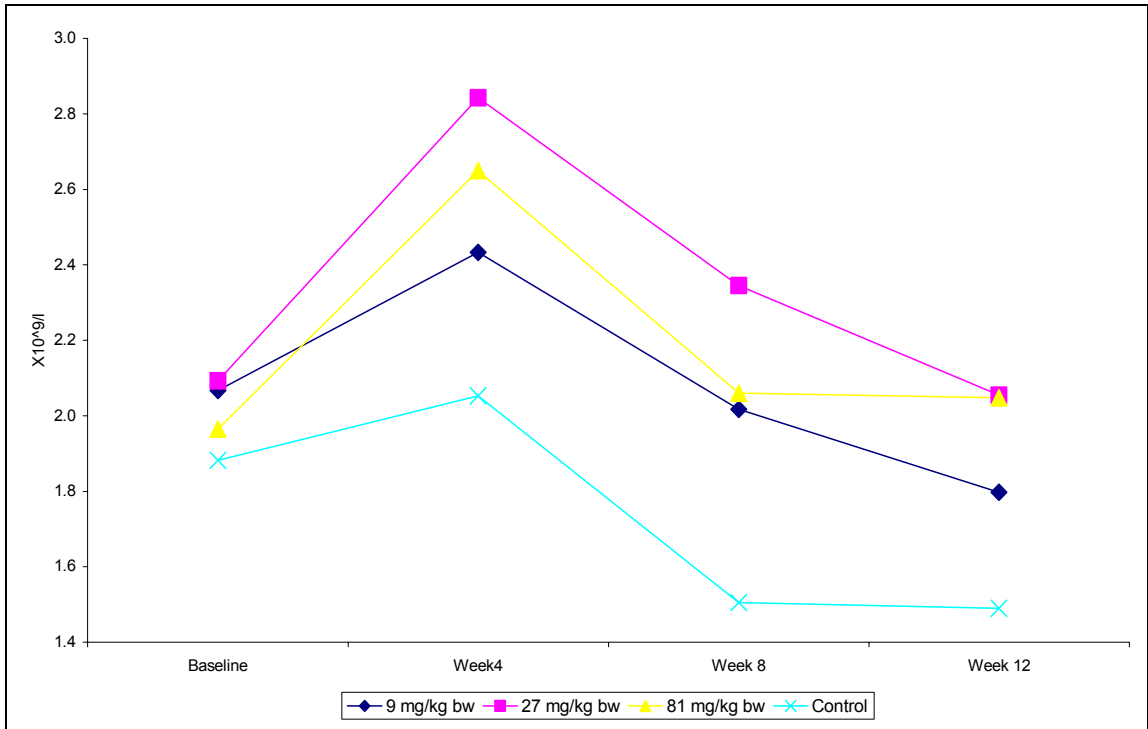


Figure 9. Neutrophils

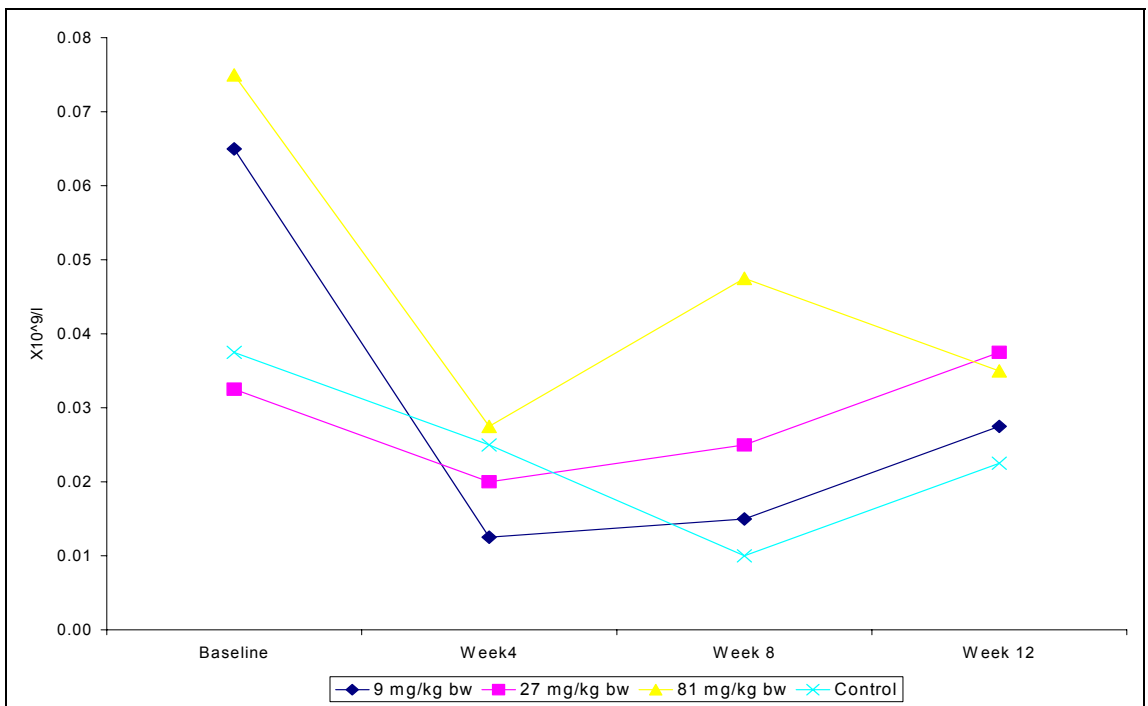


Figure 10. Eosinophils
APPENDIX I: Haematology, continued

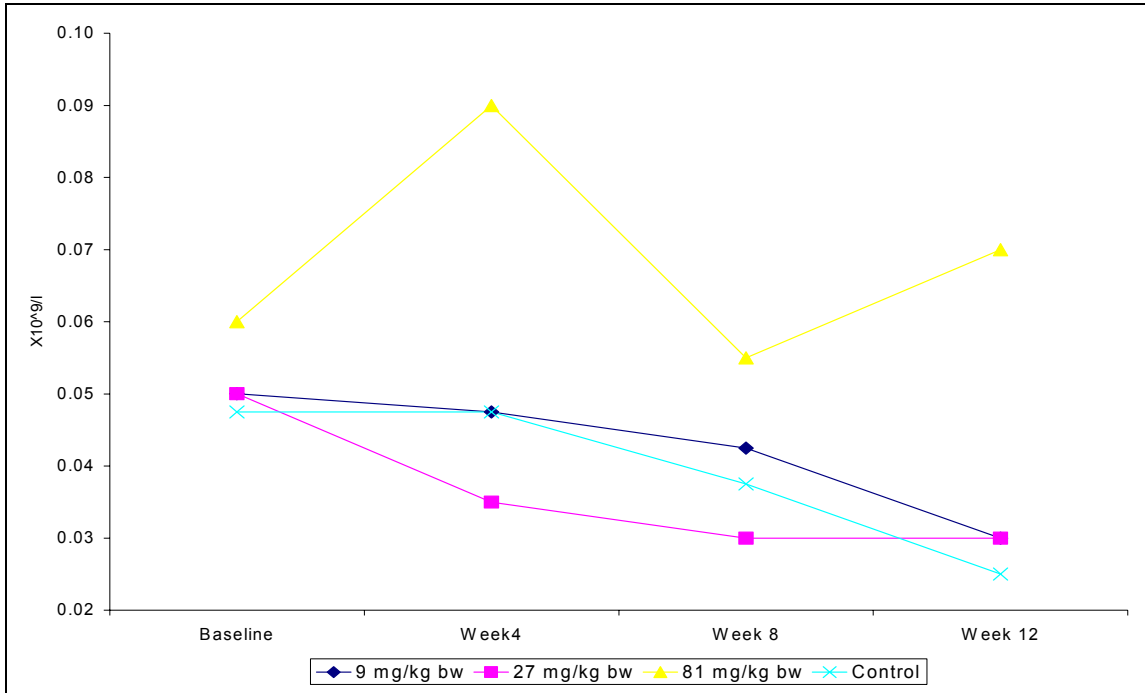


Figure 11. Basophils

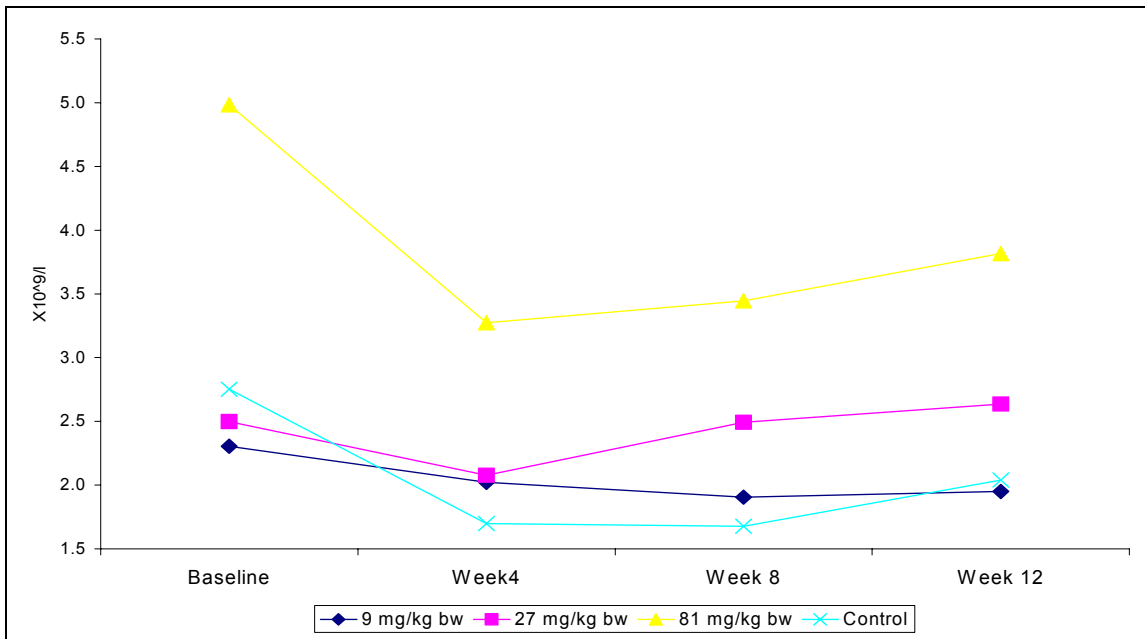


Figure 12. Lymphocytes

APPENDIX I: Haematology, continued

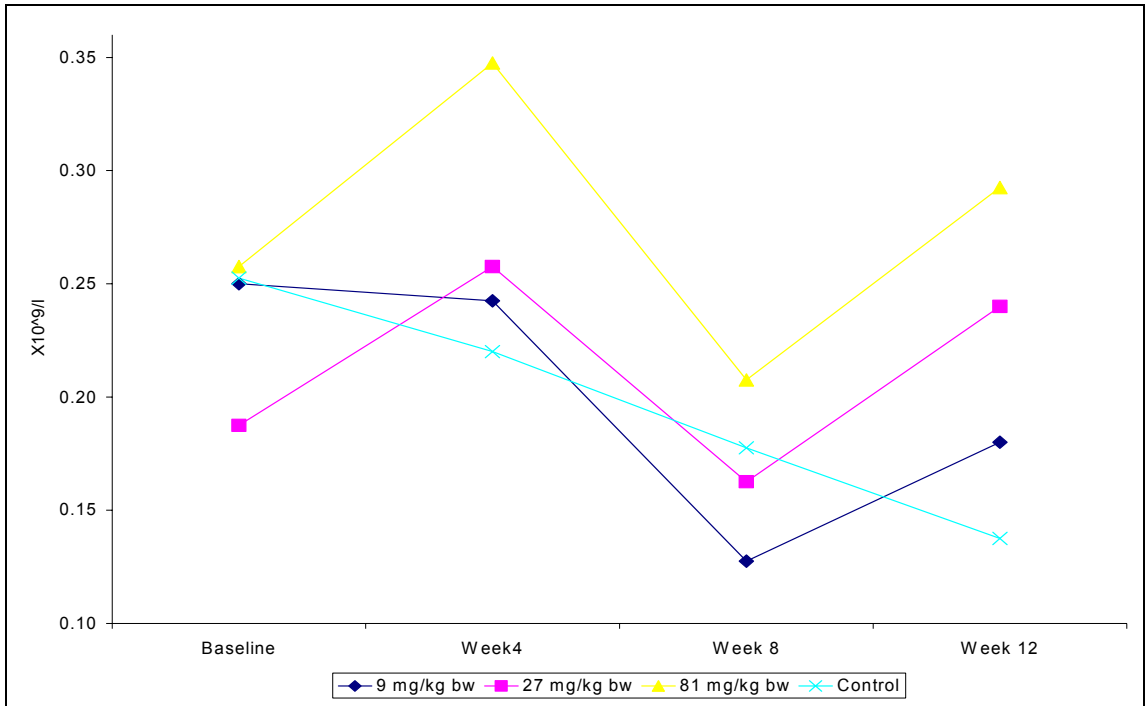


Figure 13. Monocytes

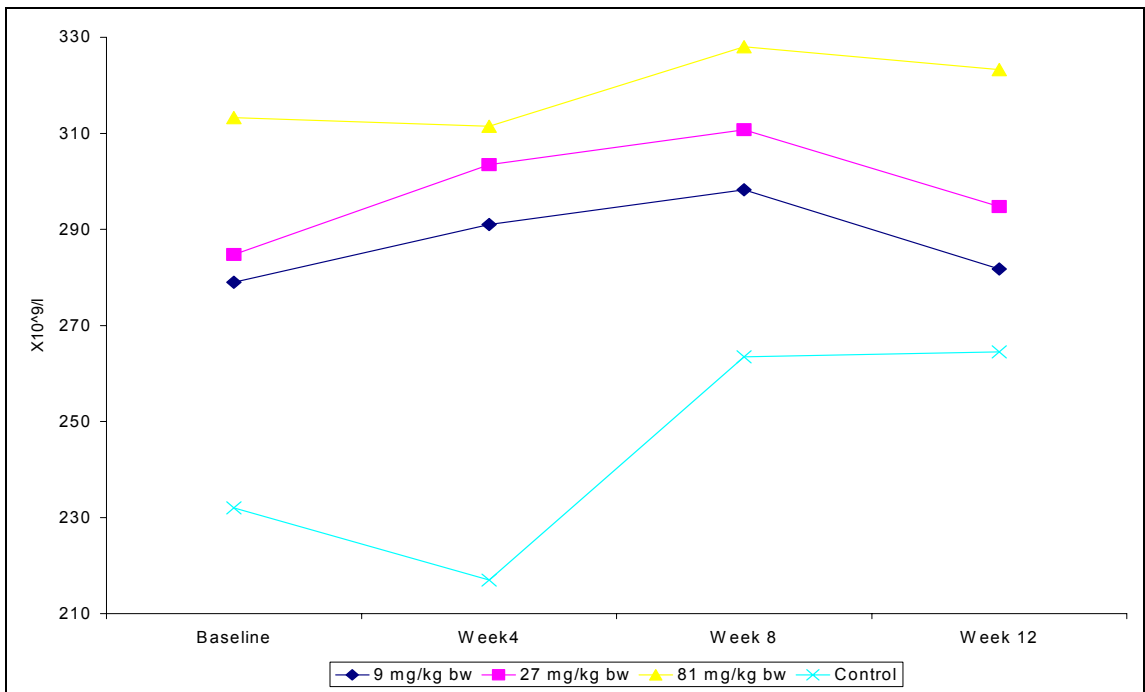


Figure 14. Platelets

APPENDIX II: Biochemistry

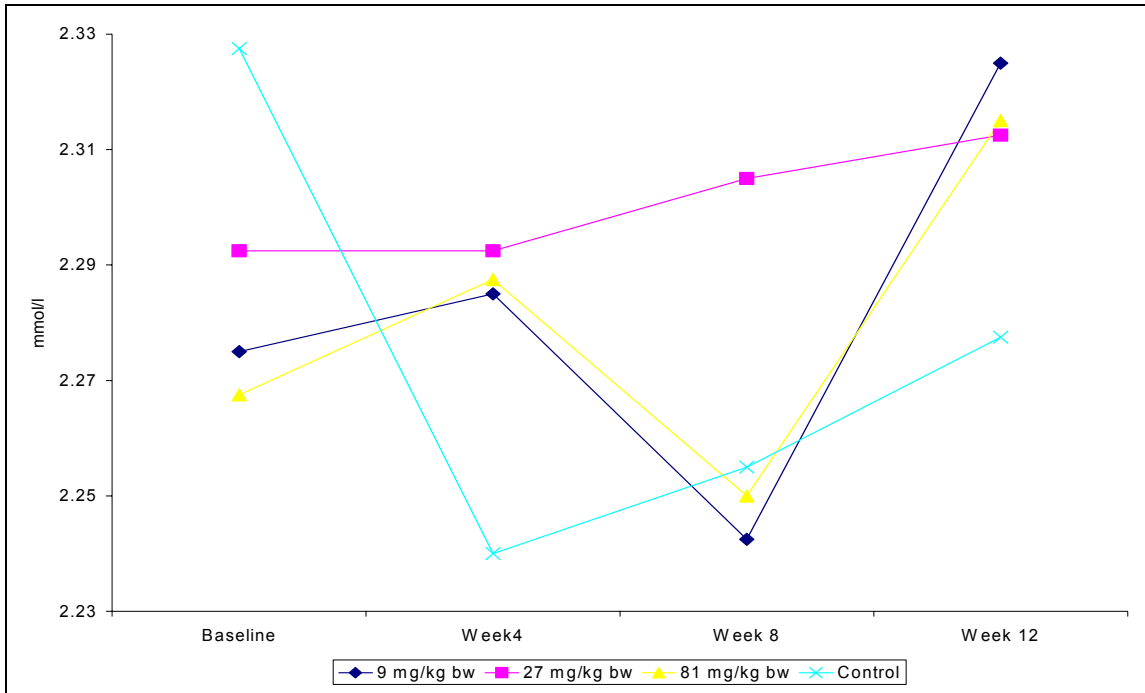


Figure 15. Calcium

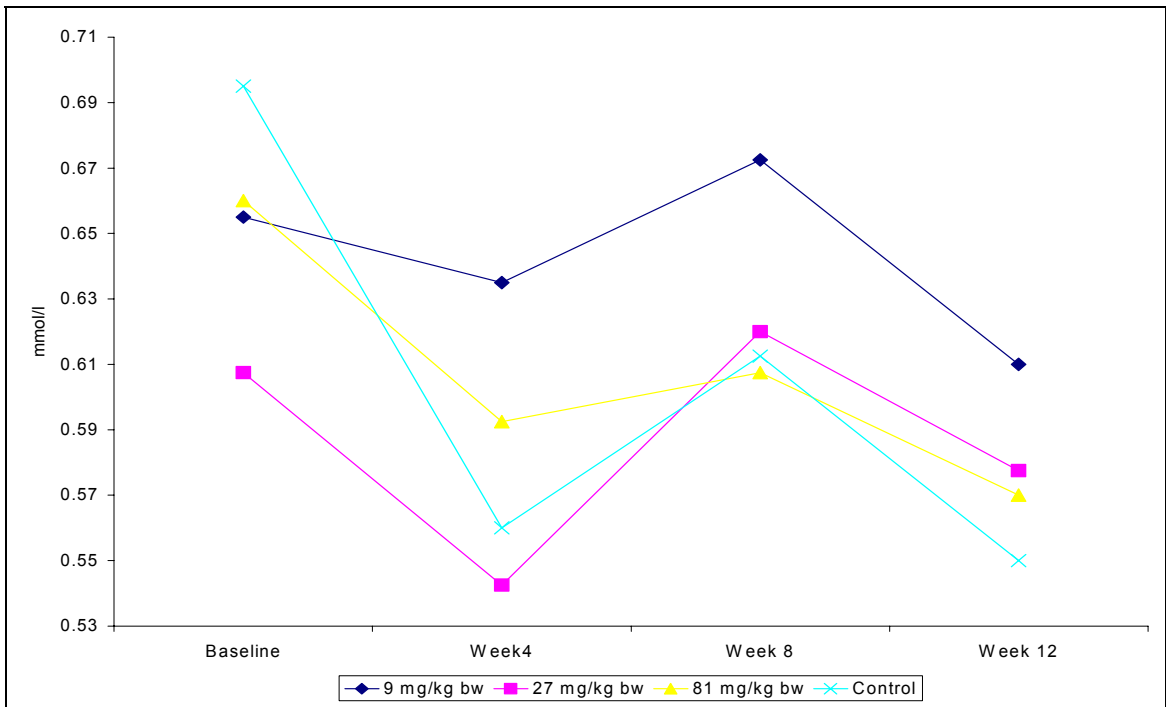


Figure 16. Magnesium

APPENDIX II: Biochemistry, continued

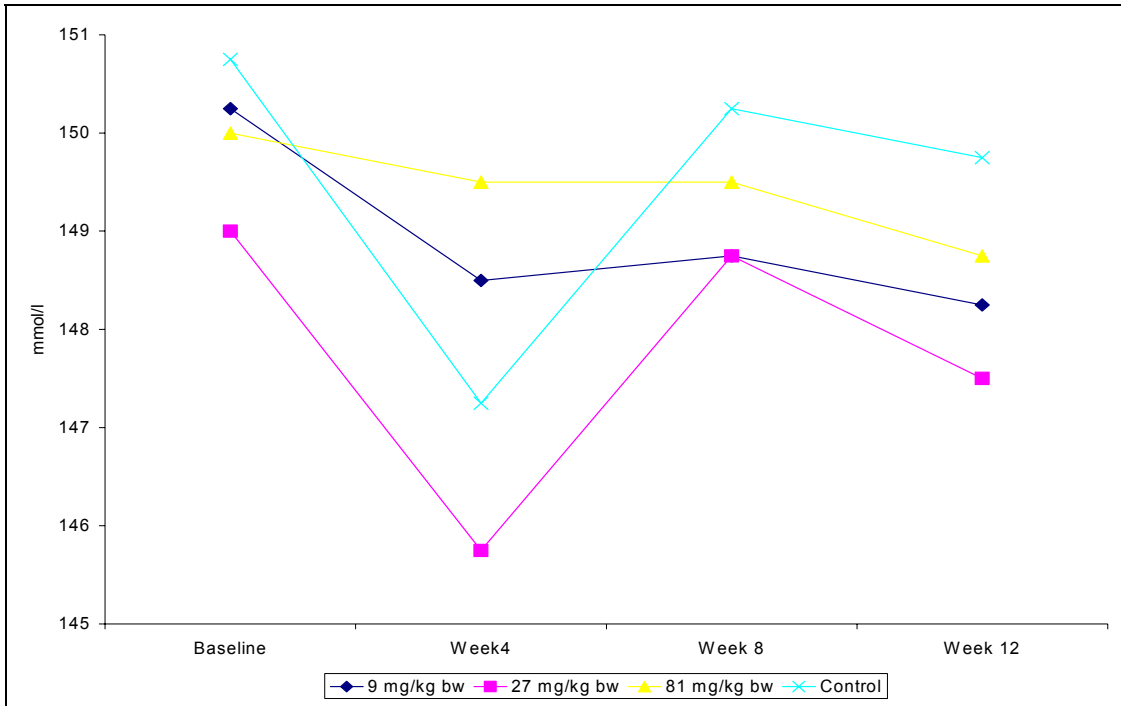


Figure 17. Sodium

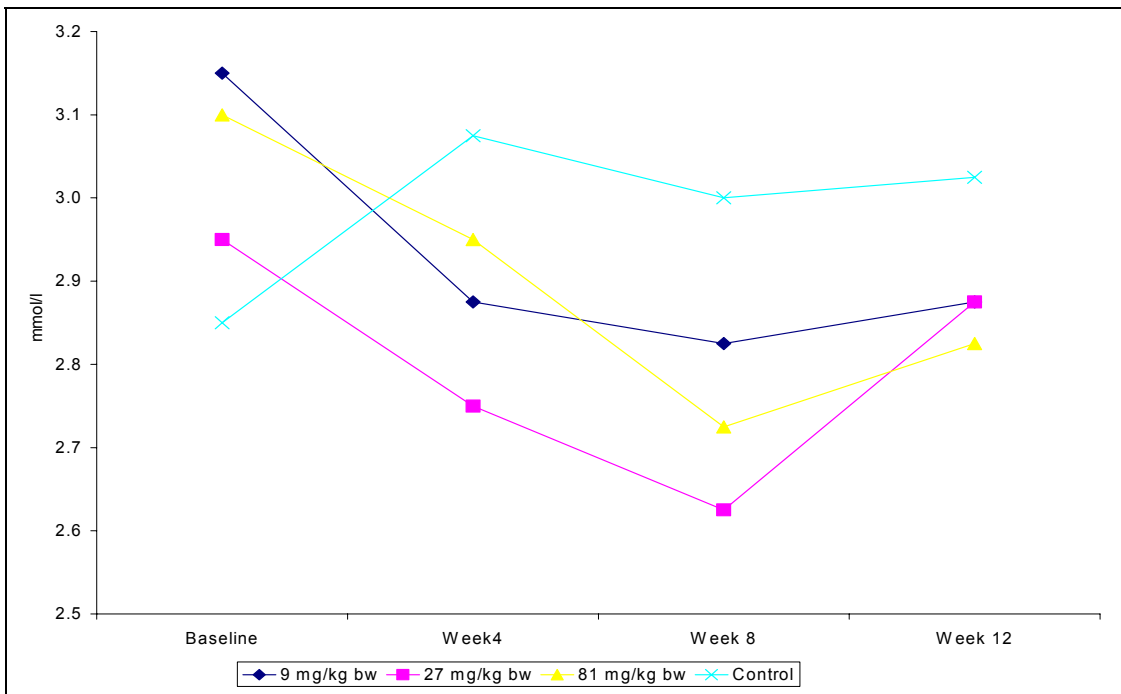


Figure 18. Potassium

APPENDIX II: Biochemistry, continued

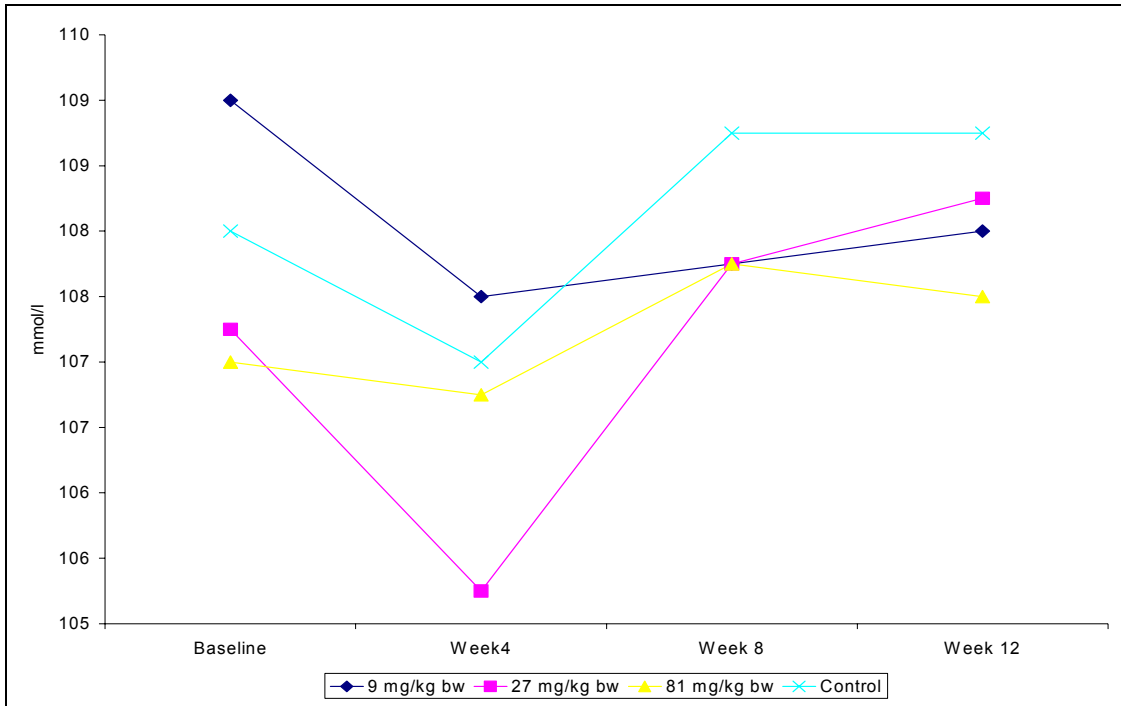


Figure 19. Chloride

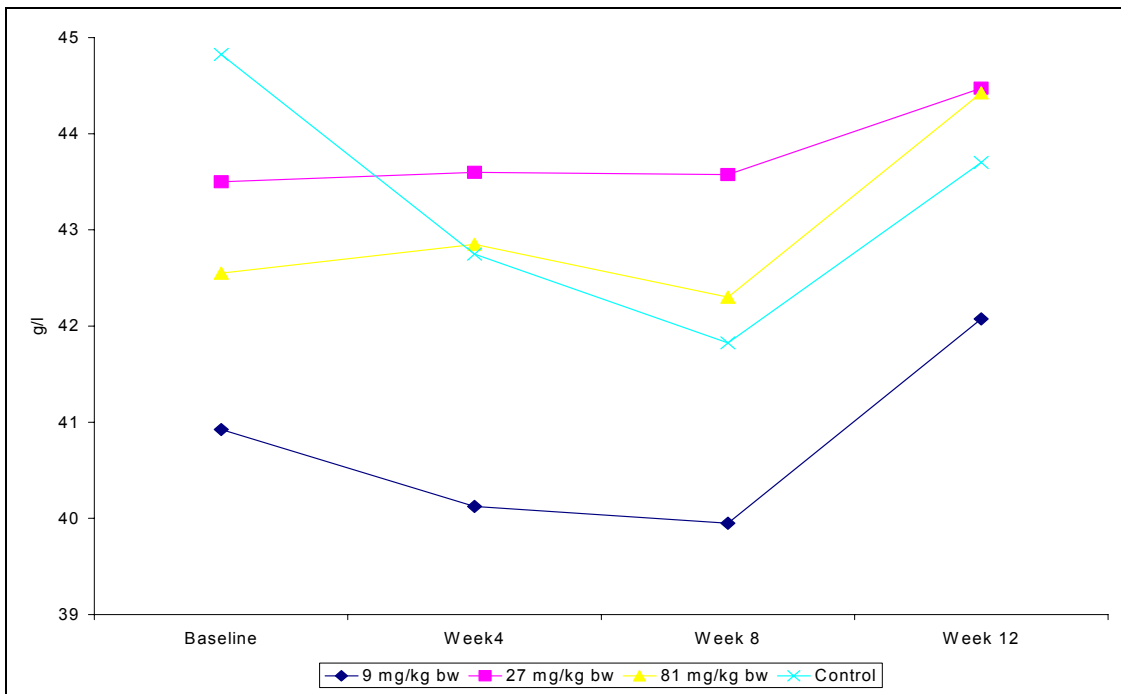


Figure 20. Albumin

APPENDIX II: Biochemistry, continued

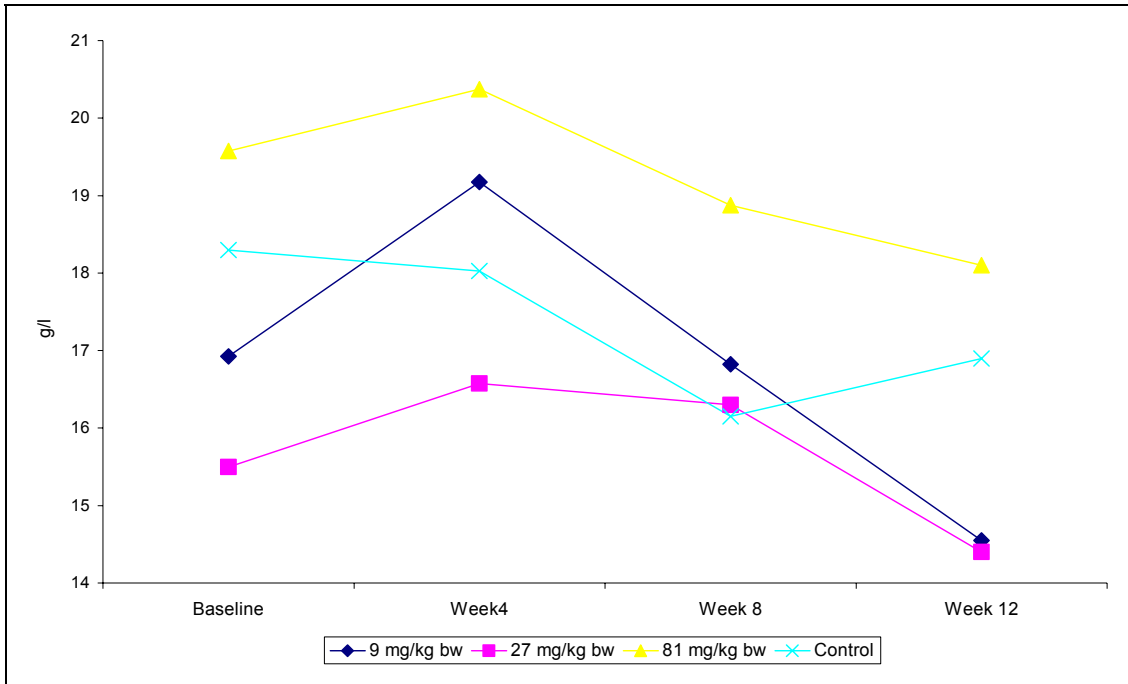


Figure 21. Globulin

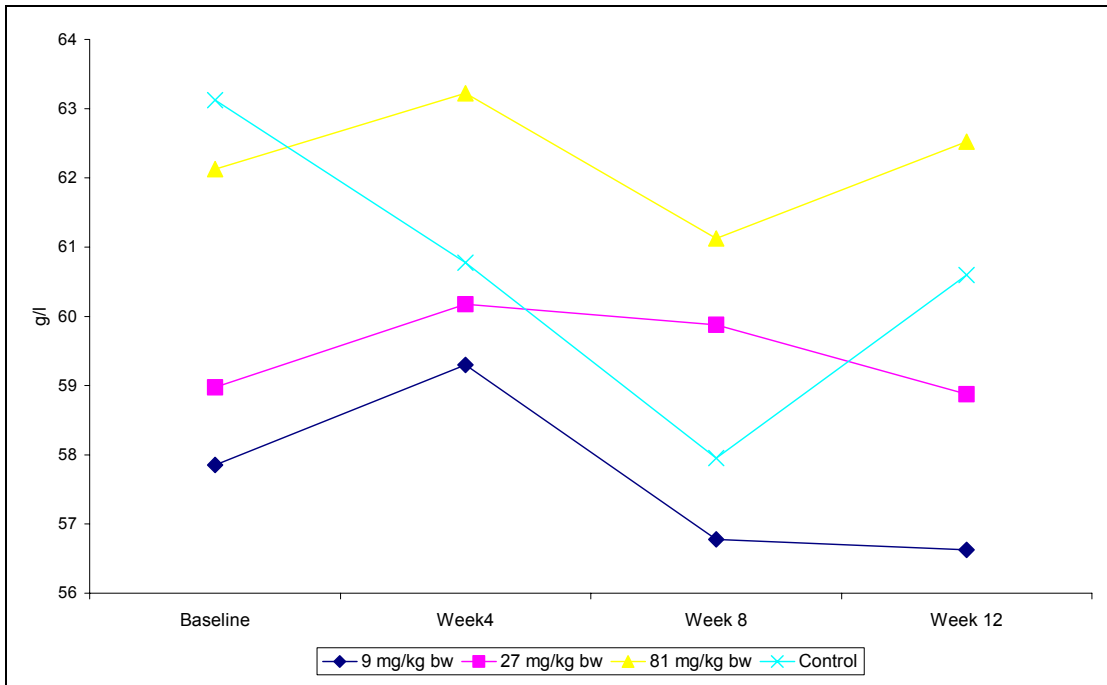


Figure 22. Total protein

APPENDIX II: Biochemistry, continued

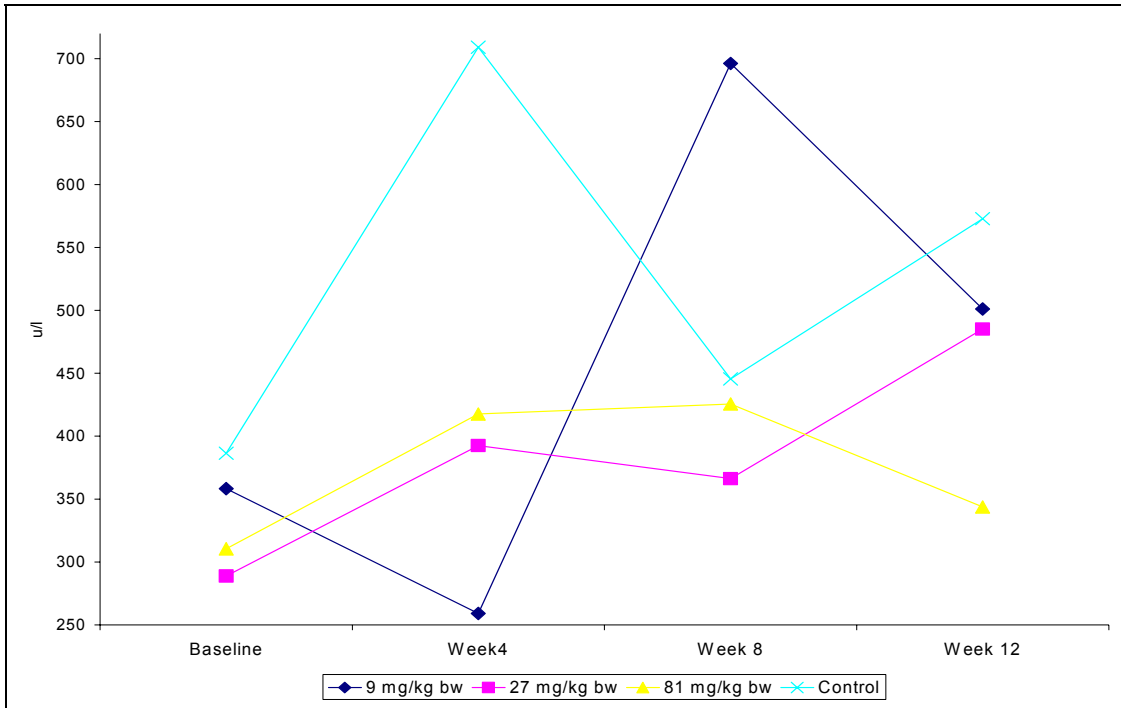


Figure 23. Creatinine kinase

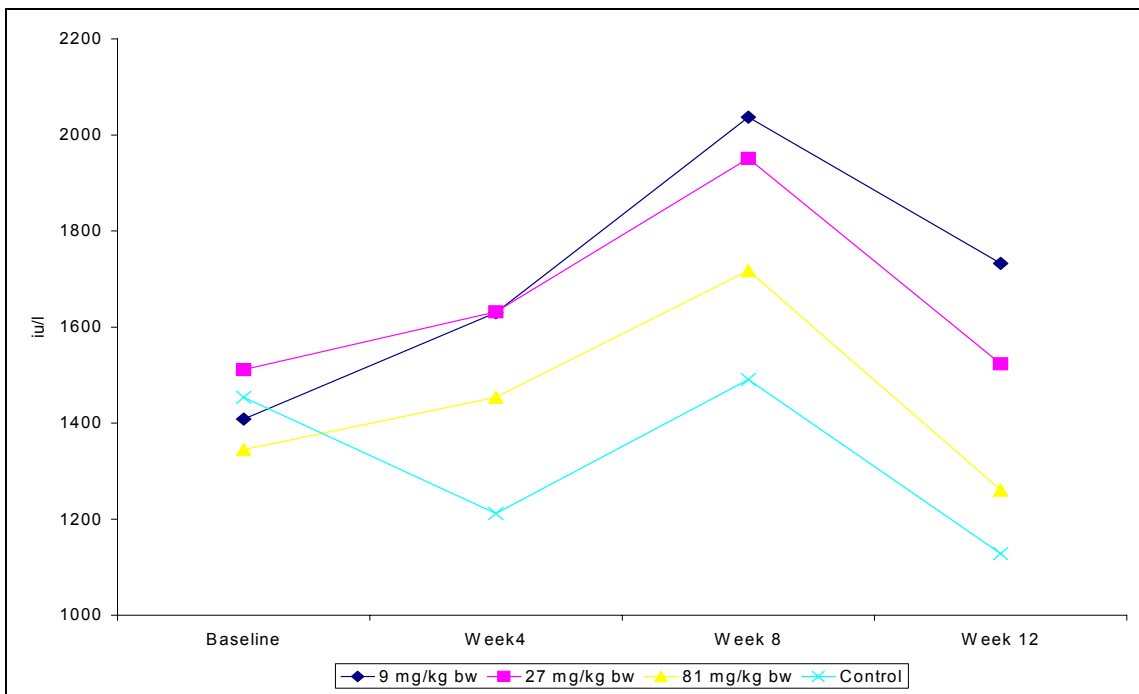


Figure 24. Lactate dehydrogenase

APPENDIX II: Biochemistry, continued

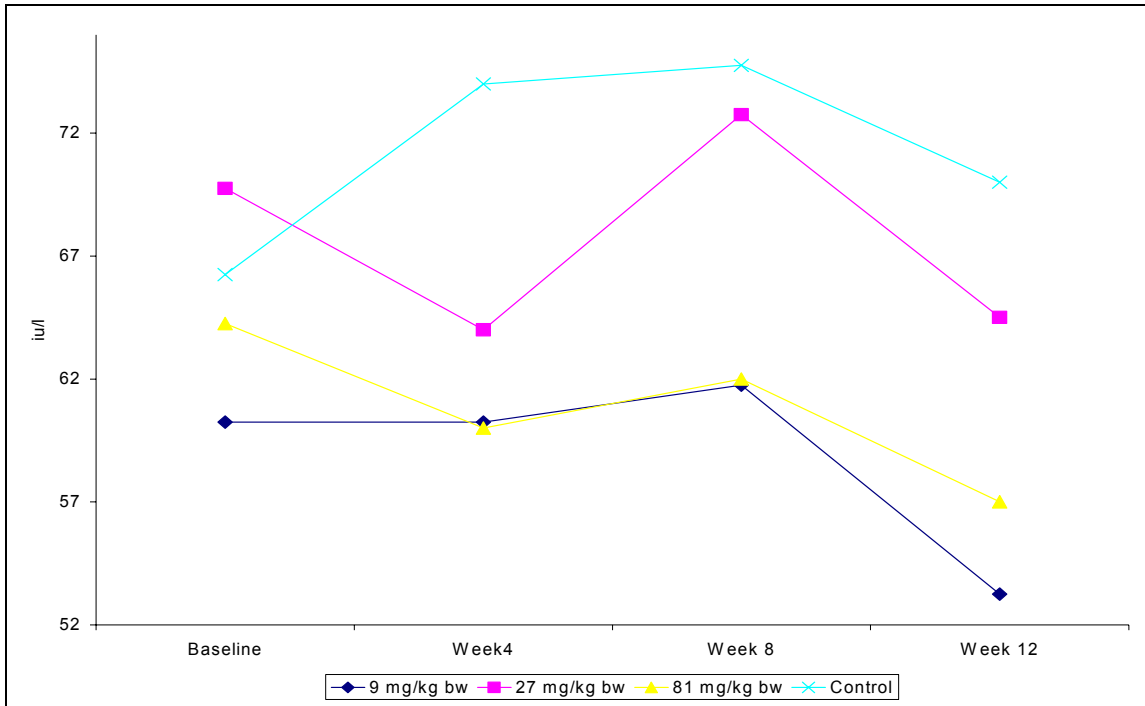


Figure 25. Gamma-glutamyl transferase

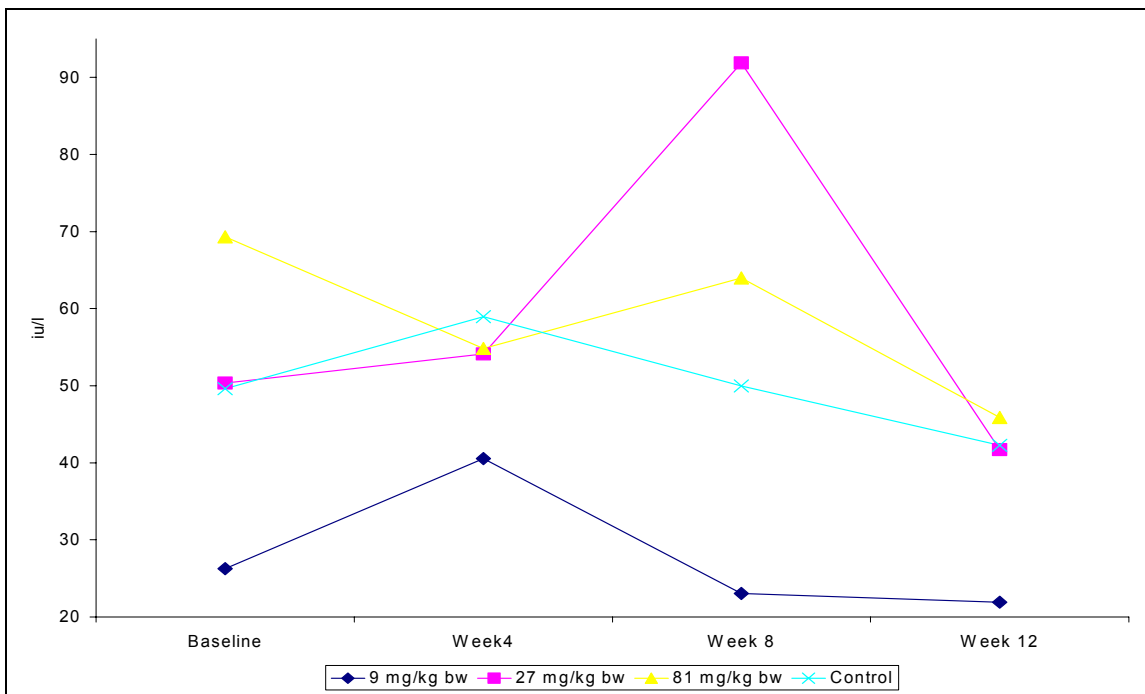


Figure 26. Alanine aminotransferase
APPENDIX II: Biochemistry, continued

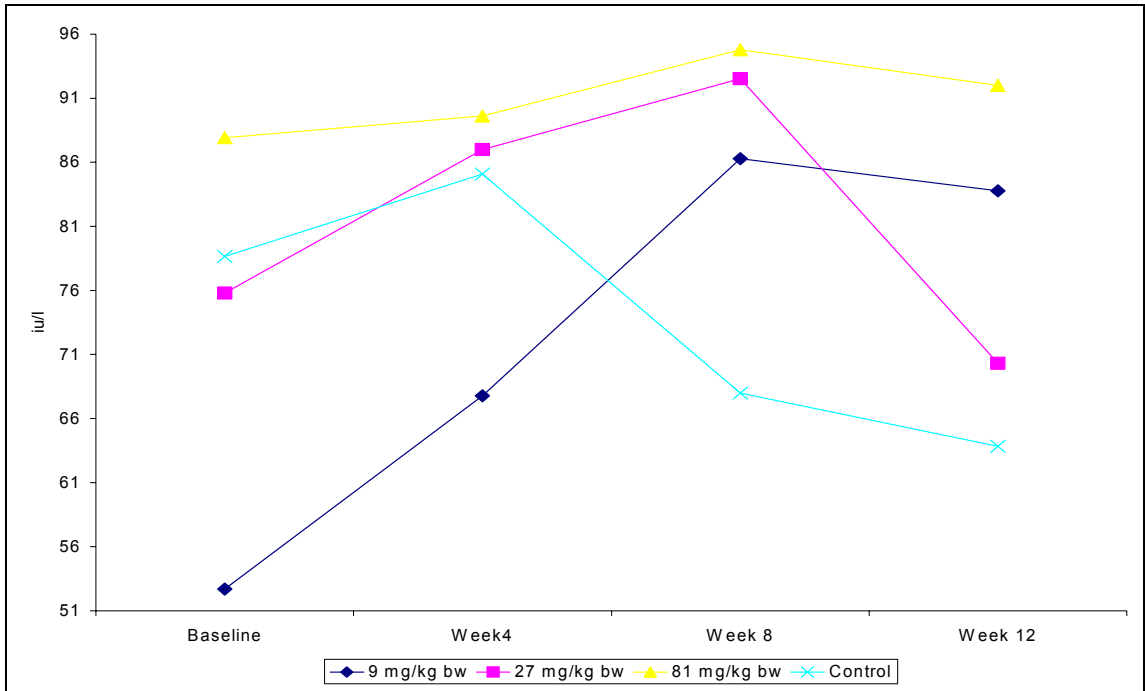


Figure 27. Aspartate aminotransferase

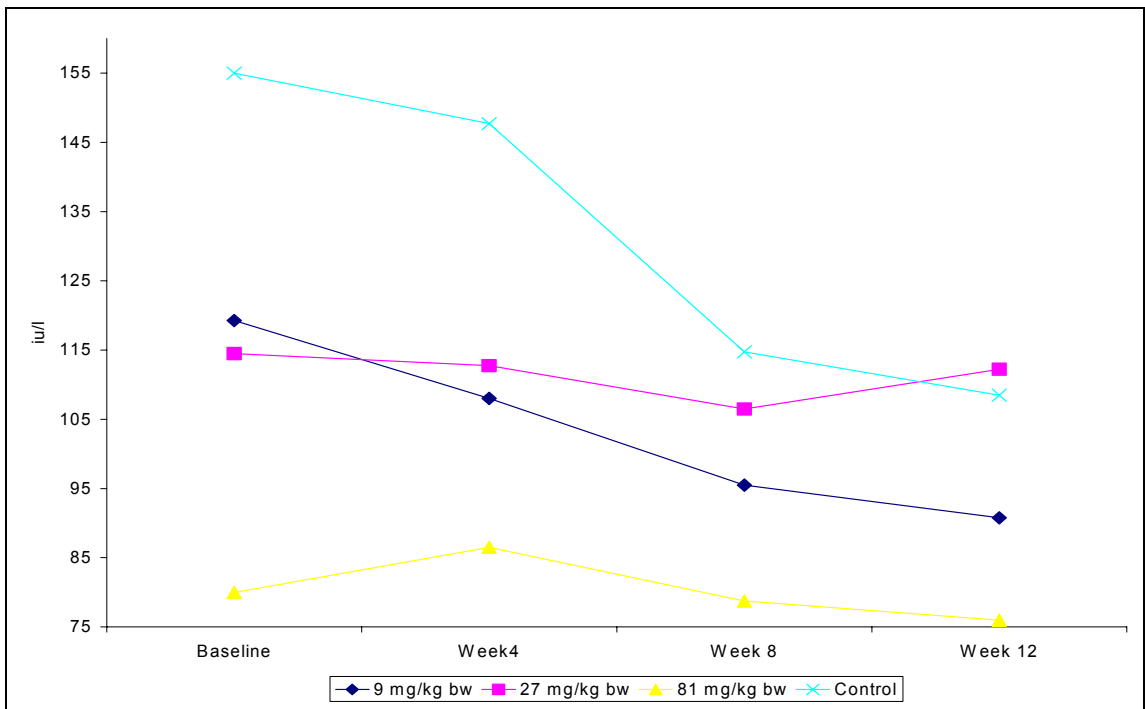


Figure 28. Alkaline phosphatase

APPENDIX II: Biochemistry, continued

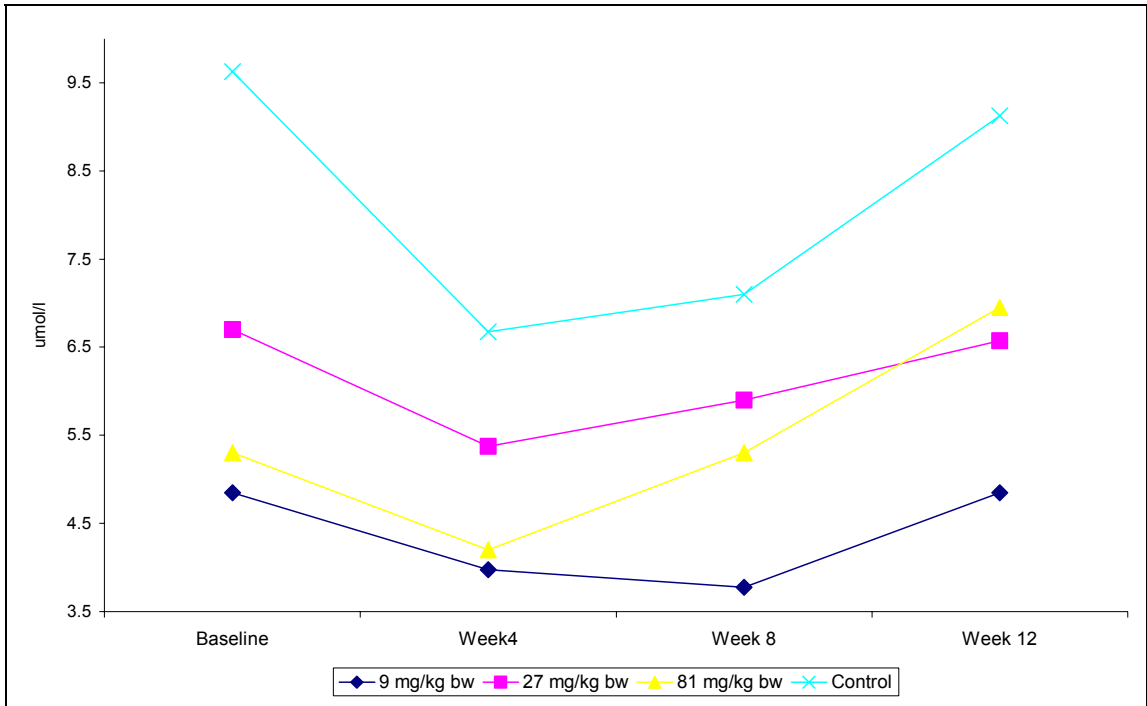


Figure 29. Total bilirubin

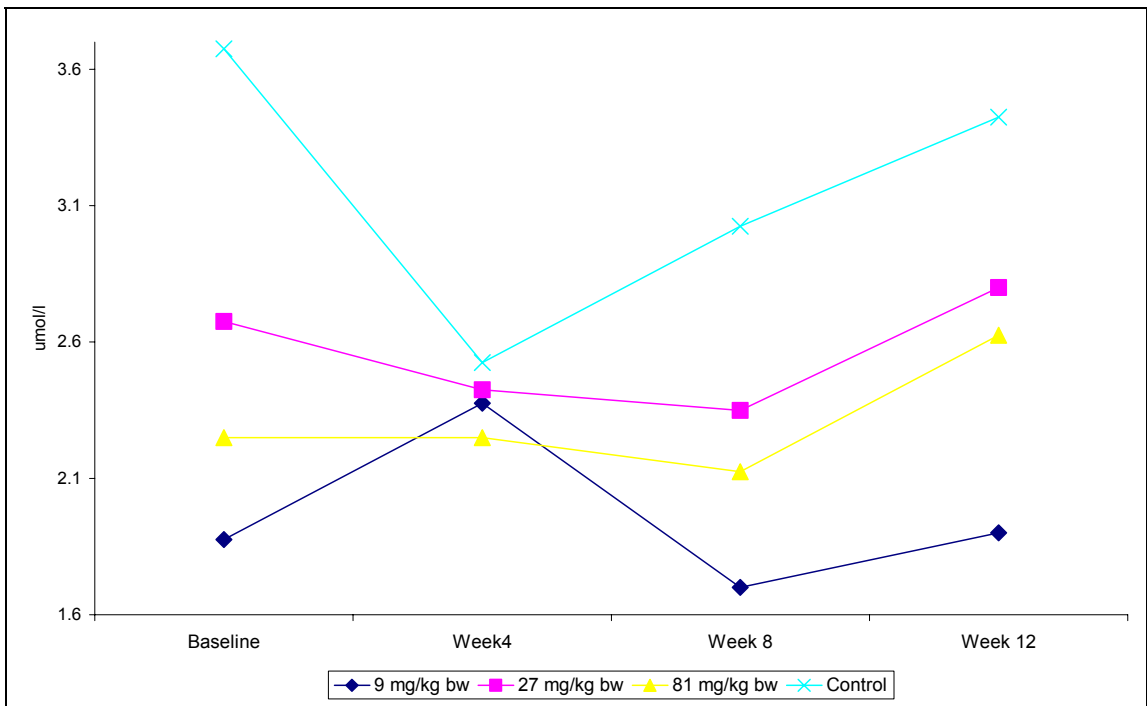


Figure 30. Unconjugated bilirubin
APPENDIX II: Biochemistry, continued

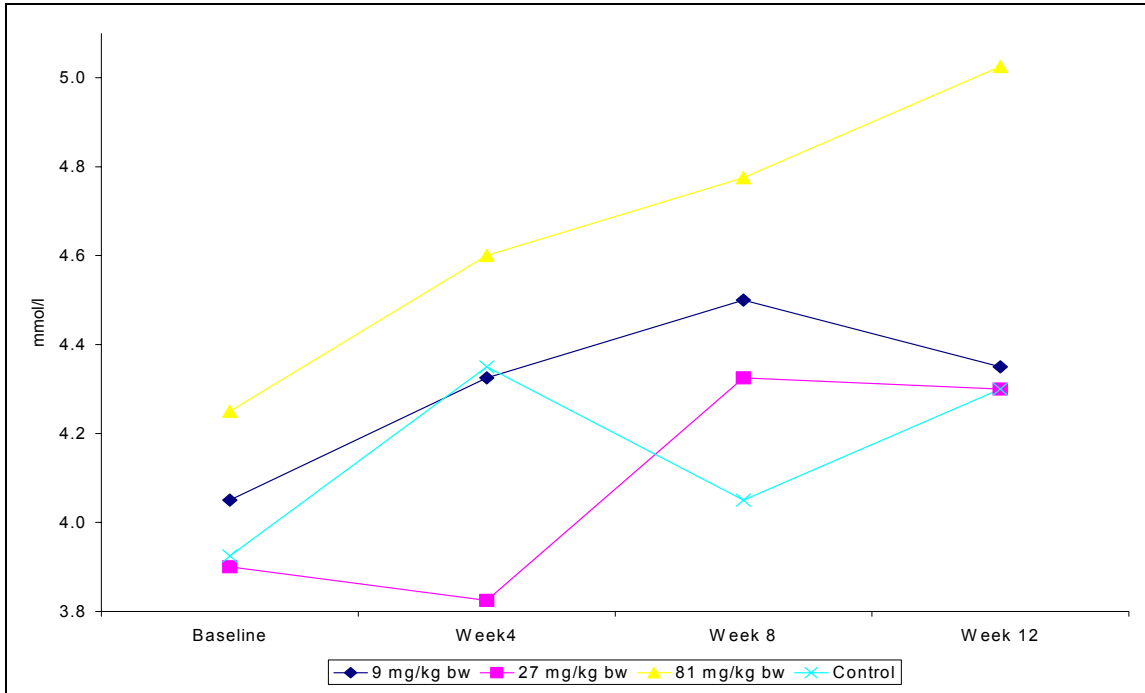


Figure 31. Glucose

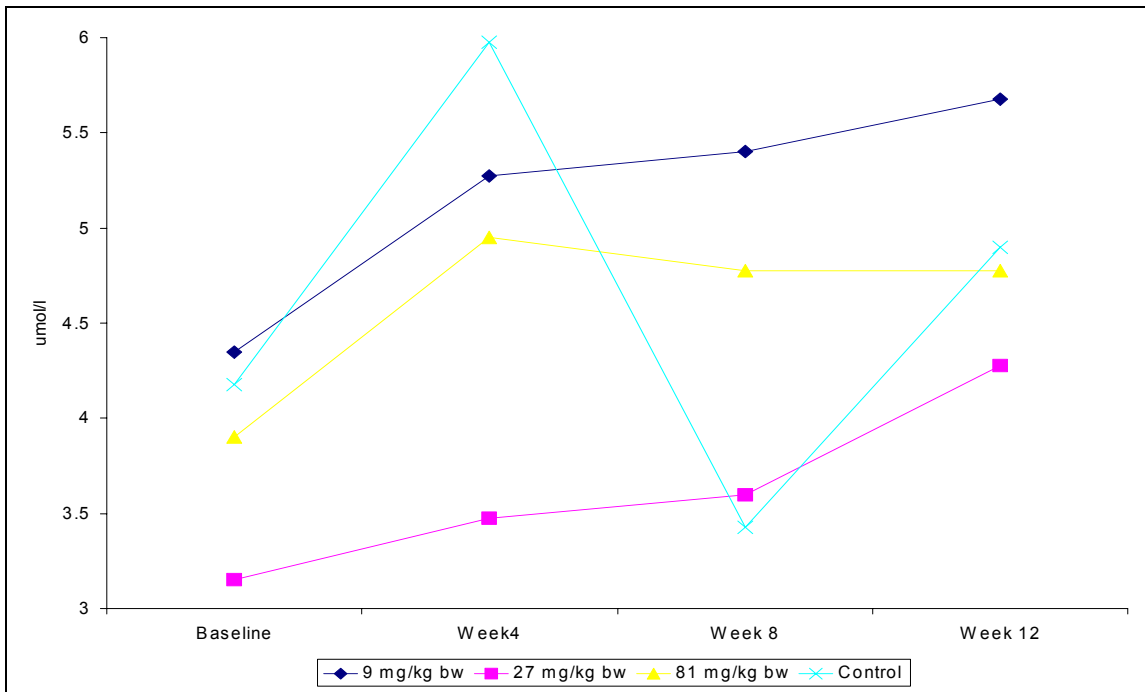


Figure 32. Urea
APPENDIX II: Biochemistry, continued

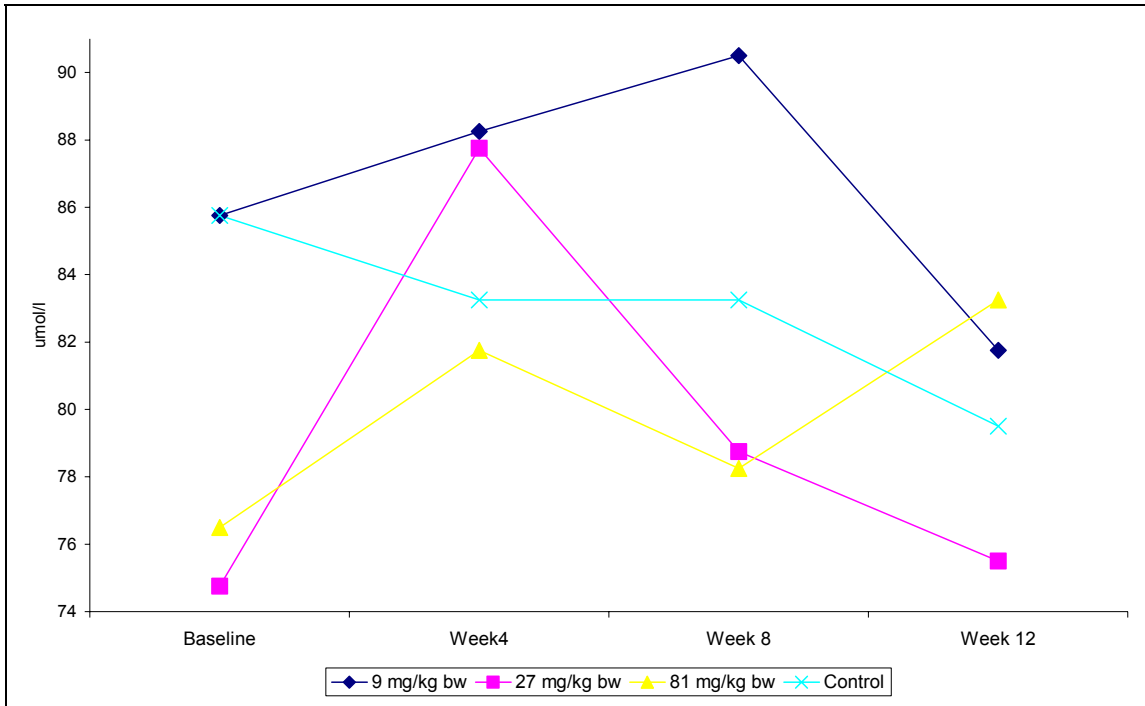


Figure 33. Creatinine

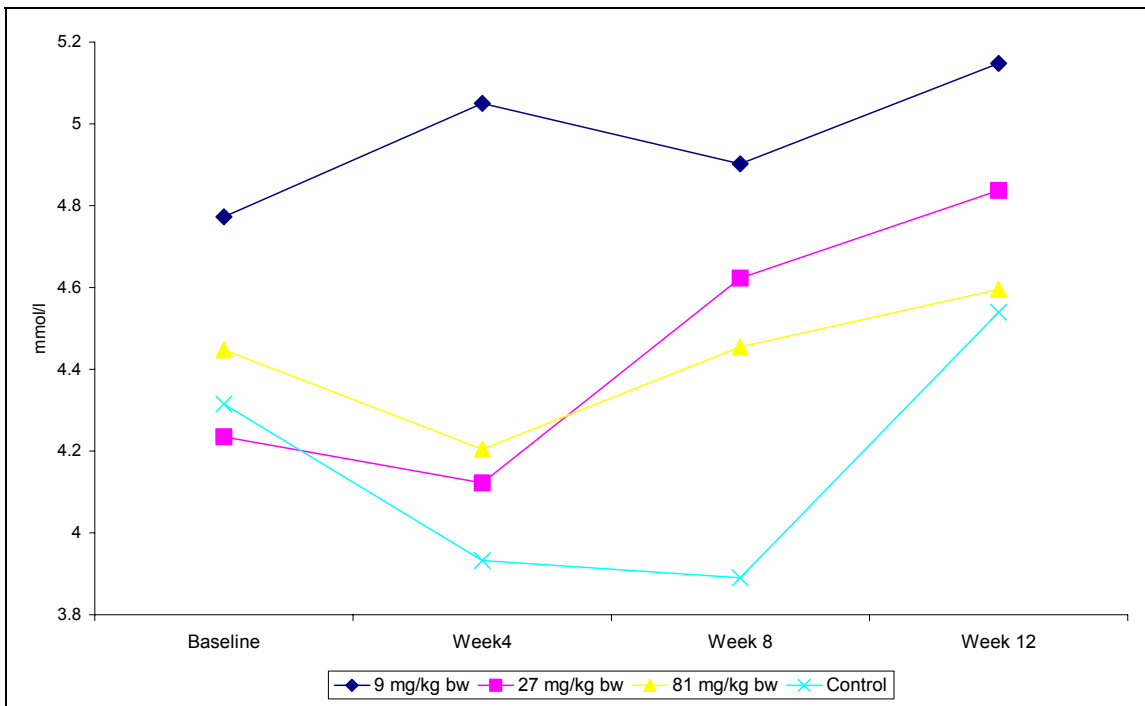


Figure 34. Total cholesterol
APPENDIX II: Biochemistry, continued

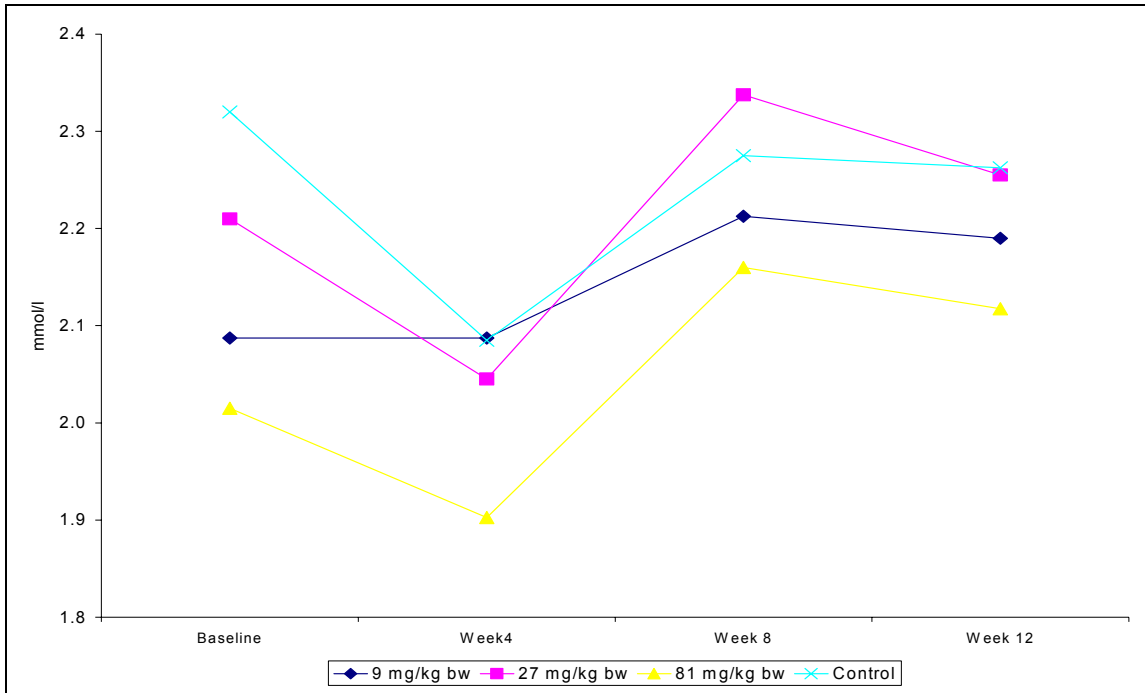


Figure 35. High density lipoprotein-cholesterol

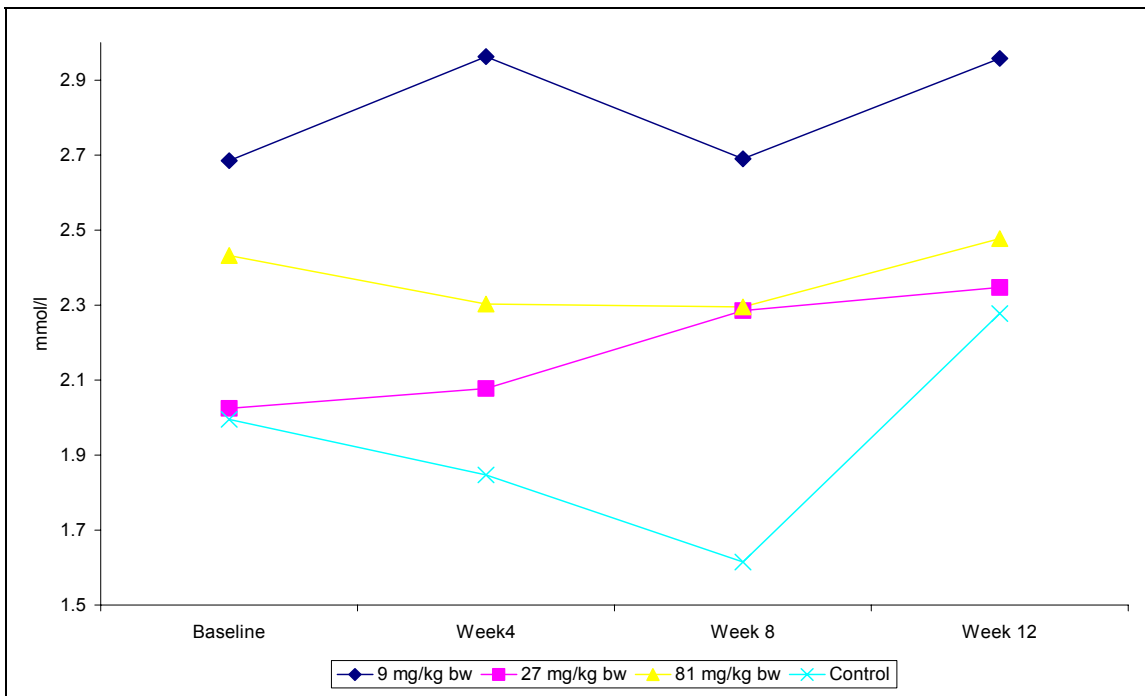


Figure 36. Low density lipoprotein-cholesterol

APPENDIX III: Physical and physiological variables

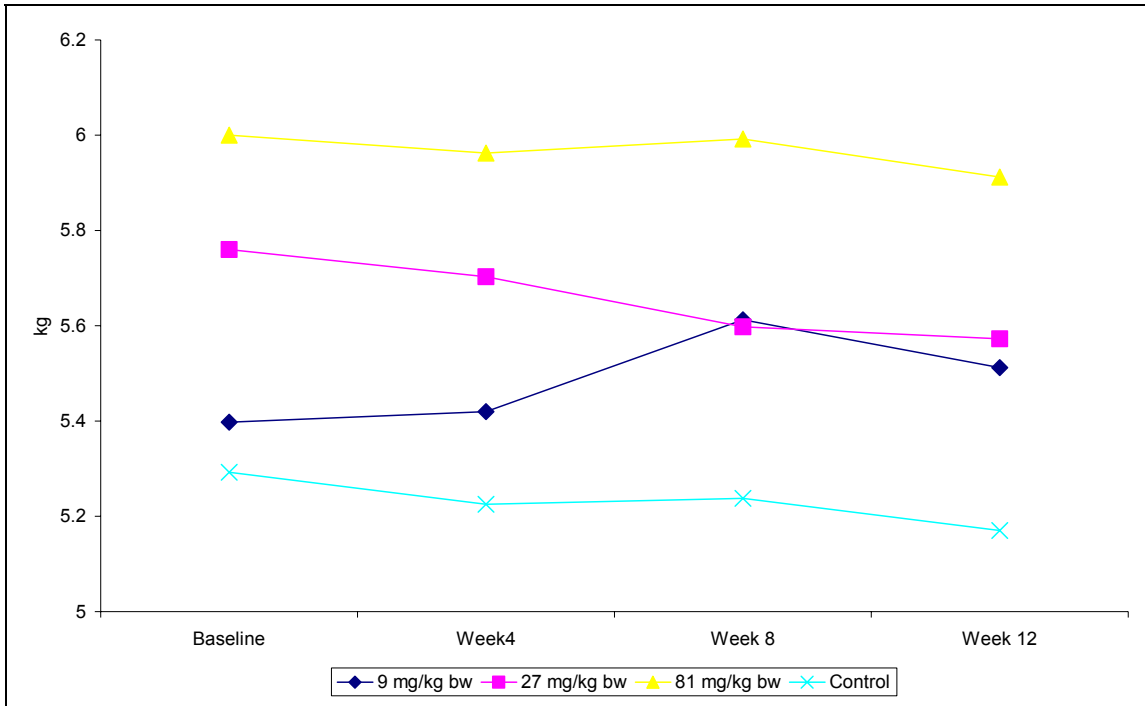


Figure 37. Bodyweight

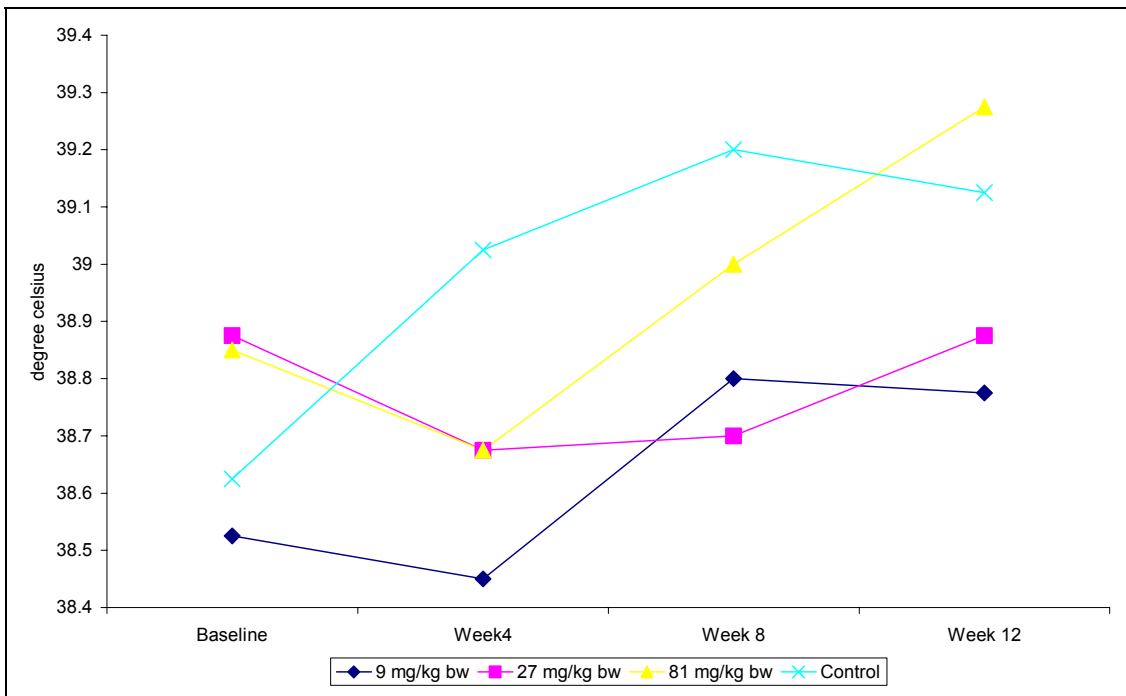


Figure 38. Body temperature

APPENDIX III: Physical and physiological variables, continued

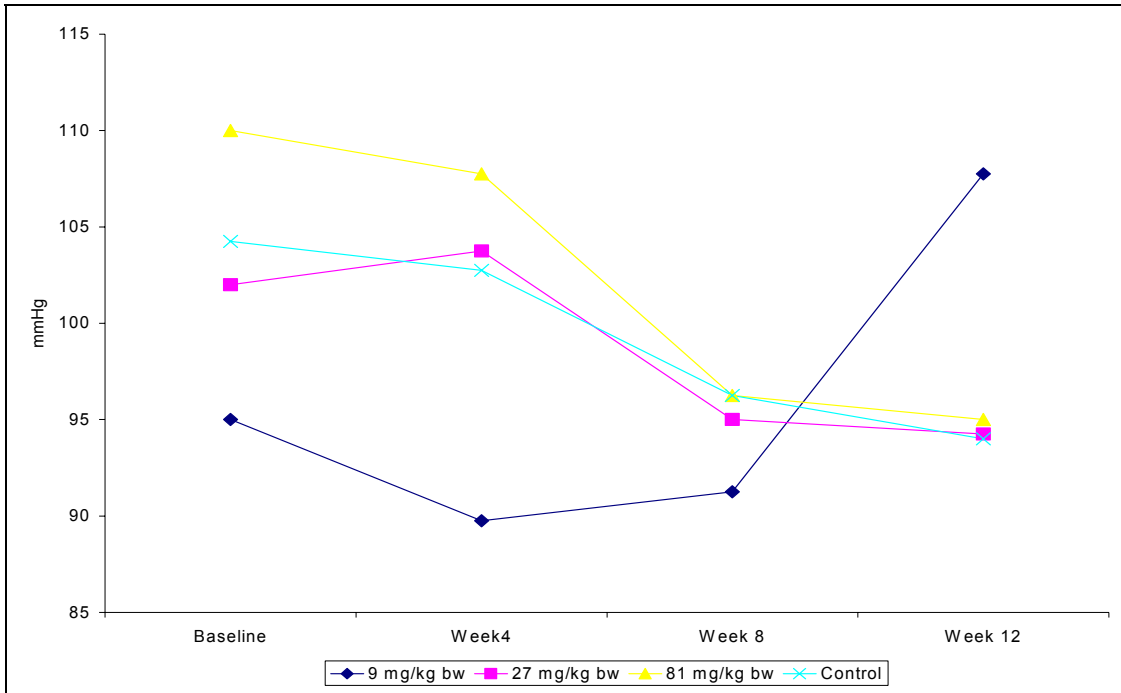


Figure 39. Systolic blood pressure

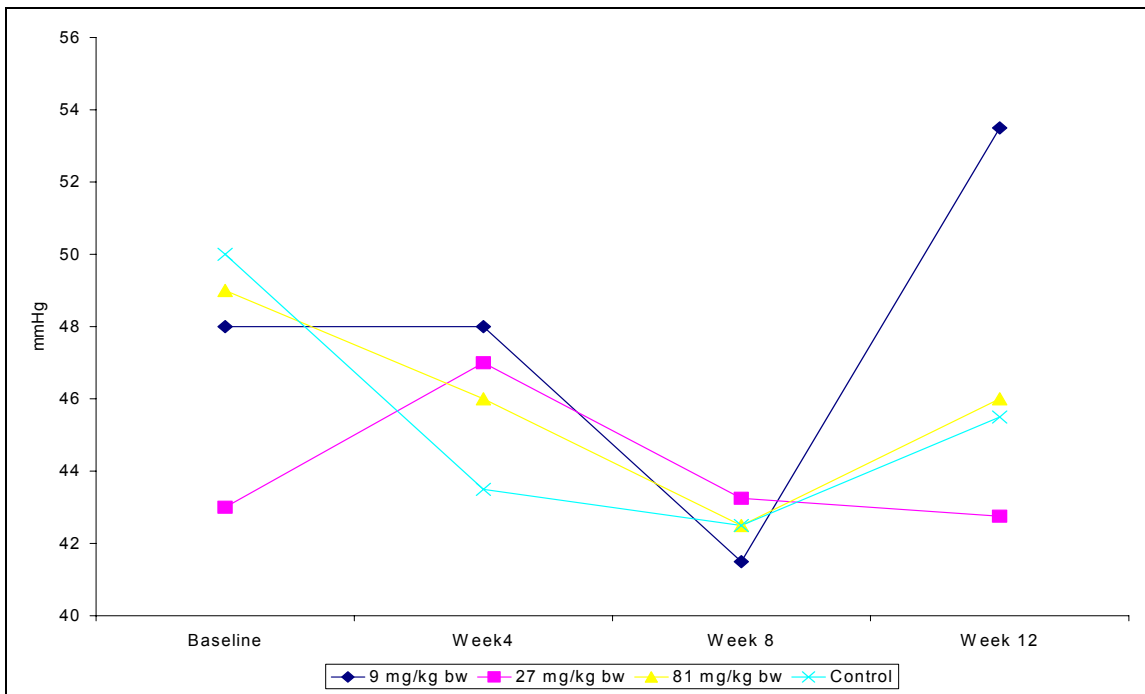


Figure 40. Diastolic blood pressure

APPENDIX III: Physical and physiological variables, continued

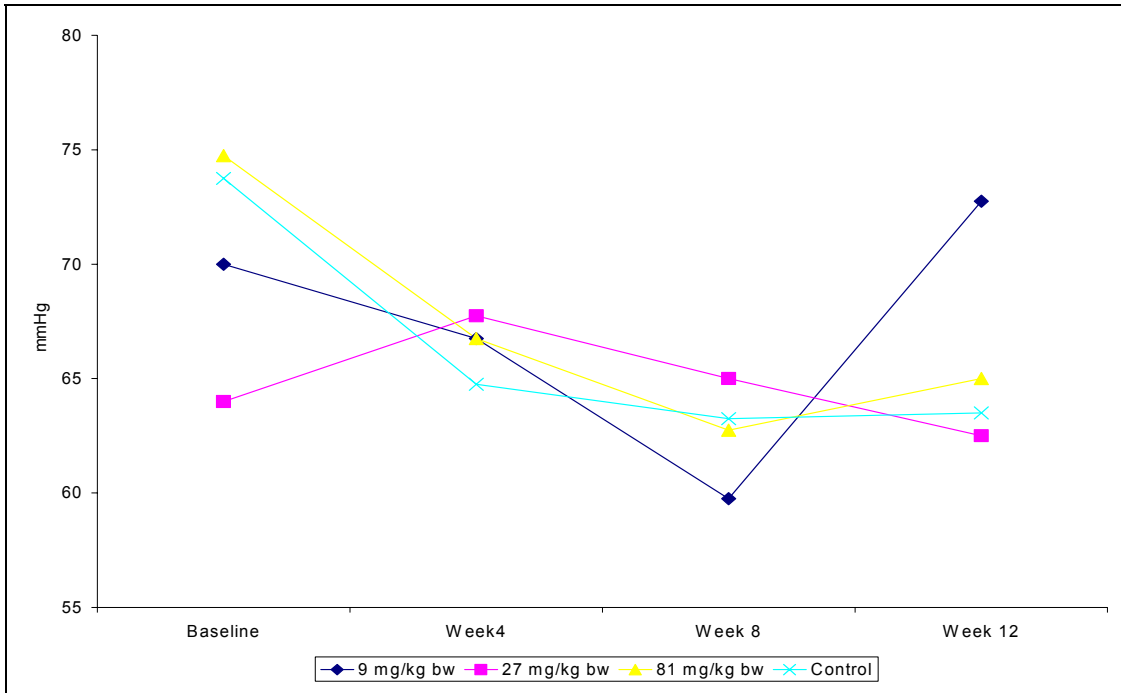


Figure 41. Mean arterial pressure

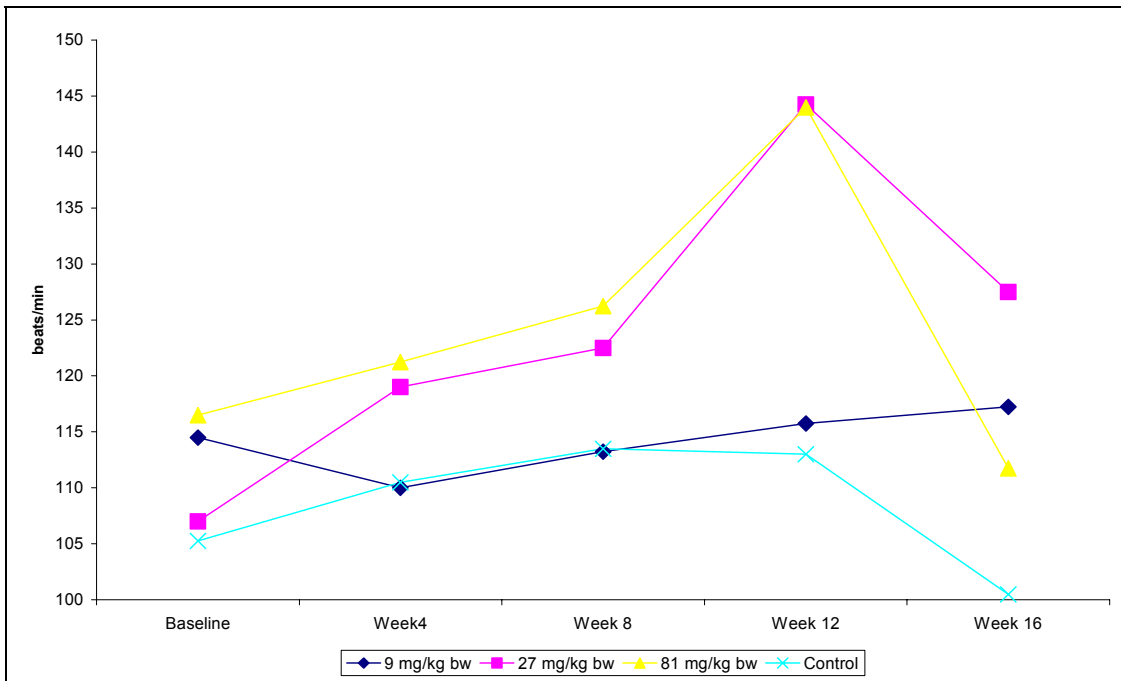


Figure 42. Heart rate

APPENDIX III: Physical and physiological variables, continued

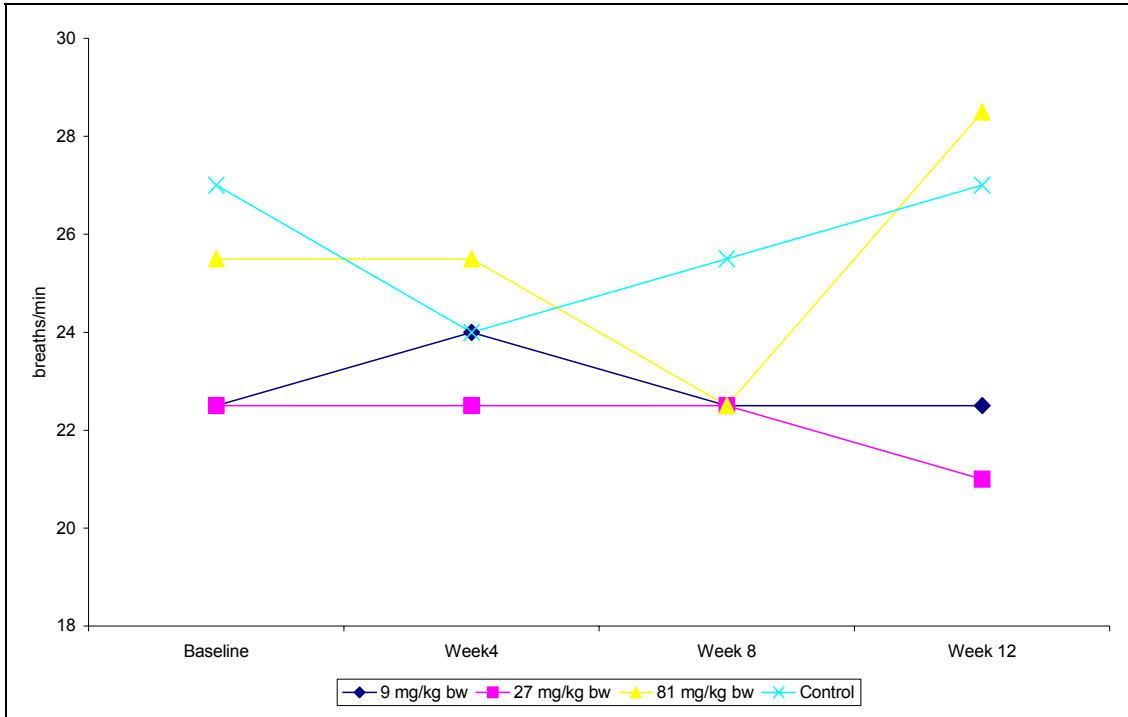


Figure 43. Respiratory rate