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Investigation of the anti-inflammatory effect of meat derived peptides using the Iodoacetate model of osteoarthritis

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Executive summary

This study investigated the effect of an Ovine Glycoprotein MLA008C6 on the development of osteoarthritis in a rat model. It was administered as a supplement in a standard rodent diet at two concentrations (0.1 & 1.0%, w/w). In addition the Precursor form of this test preparation was also supplemented in the same standard diet at the 1.0%. The test preparations were chosen as they had exhibited anti-inflammatory activity in a rat model of acute joint inflammation (carrageenan).

Young rats were fed the test preparations for three weeks prior to the initiation of osteoarthritis. At the conclusion of the experimental period each rat was sacrificed and the osteoarthritic knee joint and the contralateral normal knee joint removed for histological analyses.

It was hypothesised that the test preparations, administered as dietary supplements, would reduce the inflammation seen in the acute phase of the disease and would also have a beneficial effect on the chronic phase.

The MLA008C6 test preparation was well tolerated in the diet at the 0.1% dose but was less palatable at the high dose (1.0%, w/w). The Precursor form was the least palatable of the three test preparations. In this latter group both the daily and total food consumption were significantly reduced when compared with the Control group fed the standard unsupplemented diet.

While the animals that received the MLA008C6 (1.0% concentration) and MLA008C6 Precursor (1.0% concentration) test preparations gained the least amount of weight during the trial the differences, when compared with the Control group fed the standard diet, neither difference was statistically significant. Of the three experimental groups the animals fed the 1.0% Ovine Glycoprotein MLA008C6 Precursor form clearly demonstrated the greatest efficacy in terms of an improvement in the weight bearing capacity of the osteoarthritic limb. In these animals, the weight bearing values were above those of the Control animals for most of the trial and were very similar to the values for the Positive Control Metacam treated animals except for a short period during the acute phase where the Metacam was more effective. However, the differences between the Control and the 1.0% MLA008C6 Precursor groups were not statistically significant.

Unexpectedly the Control animals exhibited a marked and sudden improvement in the weight bearing data from Day 41 onwards. The reason for this is not clear. In all previous trials, during this chronic phase, the weight bearing values for the Control untreated animals showed a consistent and progressive decline.

For future studies it may be worthwhile to conduct a dose response experiment testing the Precursor form of the Ovine Glycoprotein MLA008C6CLA. It may also be beneficial to extend the trial out for a longer period to ascertain if the beneficial effects observed in this study are maintained in comparison with the Control groups.

Of the three test preparations investigated in this study, the one that has the greatest potential for the alleviation of osteoarthritis is the Precursor form of MLA008C6. As it was assessed at only one dose, the effect here may not be optimal.

Contents

1 Introduction and Objective

Osteoarthritis (OA) is a form of arthritis in which inflammation causes pain in the joints resulting from a variety of insults including age-related wear-and-tear, trauma, overweight, hereditary disorders, metabolic disorders or infection. In advanced stages, the patients suffer from severe pain and restriction of mobility. The consequence, in many cases, is an inability to work and often the substitution of the diseased joint with an artificial implant becomes inevitable. As cartilage tissue itself has only very limited capacity for self-renewal, the development of this disorder is chronic and progressive.

Industrial Research Ltd (IRL) has discovered several meat-derived peptides that have significant anti-inflammatory effects in a number of *in vitro* screens that measure different parameters and underlying mechanisms of inflammation. In particular, some of these fractions have been shown to have an inhibitory effect on the activated genes and enzymes that are associated with the degradation of the cartilaginous matrix of articular joints.

Objective: To determine the relative effectiveness of meat derived peptides at inhibiting joint degradation and inflammation in a model of osteoarthritis in rats. These efficacies are referenced to that produced by the anti-arthritic drug Meloxicam, which is administered to a group of rats immediately prior to the initiation of the arthritis. The arthritis model involves the injection of mono-sodium iodoacetate (MIA) into the knee joints of rats and subsequent observations on joint inflammation and weight bearing over the next 30 days.

2 Hypothesis

The supplementation of the diet with the Ovine Glycoproteins MLA 008C6 or its precursor will inhibit or prevent the development of osteoarthritis in a rat model system.

3 Outline of Experimental Methods

MLA is committed to investing in top quality scientific research, performed by suitably qualified, experienced and registered researchers and organisations. In experiments that involve livestock, MLA acknowledges that such research needs to be done under the auspices of a recognised Animal Care and Ethics Committee (AEC). The responsibility for obtaining AEC approval lies with the researcher. MLA has in the past not specifically asked for evidence that such AEC approval had indeed been obtained.

The following is a brief outline of the experimental methods. For the full detailed protocols please refer to Appendix 1.

The following test compounds were supplied by the study sponsor:
1. Ovine Glycoprotein MLA008C6
2. Ovine Glycoprotein MLA008C6 Precursor

3.1 Characterisation of the Animals.

Each test group was comprised of ten male Wistar rats, with animals being randomly assigned to groups using 5 x 5 Latin squares. The age and body weights at the start of the trial were as follows:

3.1.1 Age:

Group A: Mean: 4.6 weeks ± 0.0 (SD)
Group B: Mean: 4.6 weeks ± 0.0 (SD)
Group C: Mean: 4.6 weeks ± 0.0 (SD)
Group D: Mean: 4.6 weeks ± 0.0 (SD)

3.1.2 Weight:

Group A: Mean: 114.2g ± 26.1 (SD) (Range: 78 - 152g)
Group B: Mean: 116.0g ± 24.3 (SD) (Range: 72 - 148g)
Group C: Mean: 115.2g ± 27.5 (SD) (Range: 78 - 154g)
Group D: Mean: 114.8g ± 29.5 (SD) (Range: 68 - 150g)
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Group E: Mean: 114.8g ± 29.7 (SD) (Range: 70 - 156g)

3.1.3 Food Consumption

The test fractions were incorporated into a standard rodent chow. There were two Control groups, which received a standard rodent diet and three Experimental groups of rats. Two of the experimental groups received the MLA008C6 at two different dose levels, 0.1 & 1.0% (w/w). The third group of Experimental rats received the MLA008C6 Precursor at a dose of 1.0% (w/w). Table 1 lists the diets fed to the test groups.

Trial Group	Diet
A	Standard rodent diet
B	Standard rodent diet supplemented with 0.1% Ovine Glycoprotein MLA008C6
C	Standard rodent diet supplemented with 1.0% Ovine Glycoprotein MLA008C6
D	Standard rodent diet supplemented with 1.0% Ovine Glycoprotein MLA008C6 Precursor
E	Standard rodent diet + Metacam, 5m/kg body weight/day

Table 1: Supplements Incorporated into Animal Diets

The administration of the supplemented diets was for a three-week period before the initiation of the osteoarthritis and throughout the remainder of the experimental period. The animals were housed in pairs and fed the diets *ad libitum* along with normal drinking water.

3.1.4 Measurements

The animals were monitored daily while body weights and food consumption were measured three times weekly throughout the trial.

3.1.5 Monosodium Iodoacetate (MIA) Administration

The monosodium iodoacetate (MIA) administration was staggered over two days (Day 22 & 23) with half of the animals from each group injected each day. The MIA was made up fresh on both days using sterile saline to prepare a 40mg/ml solution. The animals were anaesthetized and the MIA administered as a single 25µl injection through the infrapatellar ligament of the right knee of each animal. The left contralateral knee of each animal was injected with 25µl of sterile saline.

3.1.6 Hind Foot Weight Bearing Measurements

On Day 19 the hind foot weight distribution of all animals was measured using an incapacitance tester to obtain baseline readings. As the MIA administration was staggered so too were the hind foot weight distribution measurements which were conducted on Days 23 & 24, 26 & 27, 29 & 30, 32 & 33, 36 & 37, 39 & 40, 43 & 44, 46 & 47, 50 & 51. The changes in the weight distribution

were calculated as a percentage of weight on the right osteoarthritic foot using the following formula in Pomonis *et al* (2005), Pain 114: 339-346.

$$\% \text{ weight on right limb} = \left[\frac{\text{weight on right limb}}{\text{weight on right limb} + \text{weight on left limb}} \right] \times 100$$

3.2 Amendments

There were no intended changes to the study plan after the study initiation date.

Trial Group
A
B
C
D
E

Standard rodent diet supplemented with 1.0% Ovine Glycoprotein MLA008C6 Precursor
Standard rodent diet + Metacam, 5m/kg body weight/day

Diet
Standard rodent diet
Standard rodent diet supplemented with 0.1% Ovine Glycoprotein MLA008C6
Standard rodent diet supplemented with 1.0% Ovine Glycoprotein MLA008C6

3.3 Deviations

In the original study plan it was intended to analyse the serum from each animal ofeach group for the presence of the cytokines, Tumour Necrosis Factor-α (TNF-α) and Interleukin-1β (IL-1β). However, the client subsequently decided that they did not want these analyses carried out. The serum was however, kept and stored frozen at -200C.

3.4 Conclusion of Experimental Study

The animals were anaesthetized at the end of the trial on Days 52 and 53 and blood taken via cardiac puncture, dispensed into serum separating tubes and allowed to clot. The resulting serum was removed, dispensed into cryovials and frozen at -200C. The left and right knee joints from all animals was excised and fixed in 4% buffered formalin.

3.5 Statistical Analyses

In order to assess whether the observed differences between groups with regards to the various parameters measured during the trial, the relevant data was subjected to the following statistical analyses.

1. The standard deviation, the standard deviation percentage, the standard error of mean and the standard error of mean percentage were calculated.
2. An assessment of preliminary statistical significance for the weight bearing using independent Student t-tests at $\alpha \leq 0.05$ was conducted at individual time points. This was based on the comparison of mean values of small populations (Table 2).

The difference between the values for each of the experimental groups and the control group was evaluated.

As well ANOVAs for average percentage body weight changes and for average food consumption were undertaken.

An ANOVA of the raw weight bearing data was also performed.

T-Test Formula

$$T = \frac{m_1 - m_2}{\sigma} \sqrt{\frac{(n_1 n_2)}{(n_1 + n_2)}}$$

Where $$\sigma^2 = \frac{(n_1 SD_1^2) + (n_2 SD_2^2)}{(n_1 + n_2) - 2}$$

m_1 = Mean of the 1st population
m_2 = Mean of the 2nd population
n_1 = Number of values in the 1st population
n_2 = Number of values in the 2nd population
SD_1 = Standard deviation of the 1st set of population values
SD_2 = Standard deviation of the 2nd set of population values

Reference: Statistical Methods for Technologists, Paradine, C.G. and Rivett, B.H.P. (eds), Chapter VI, Small Samples, Student's t, Variance Ratio, pp97-123 The English Universities Press Ltd, London, 1968.

Table 2. The formula for the t-test employed. The T value obtained from the formula was then entered into an online statistical software program (GraphPad: http://www.graphpad.com/quickcalcs/ttest1.cfm) and a two-tailed p value obtained.

For the statistical analysis of the incapacitance data at individual time points, the Excel Two Sample T-Test (Unequal Variance, Two Tailed) was used. When compared with the above formula, the p values were found to be almost identical.

4 Results and Discussion

4.1 Summary of Results:

The animals whose diet had been supplemented with 1.0% of the Ovine Glycoprotein MLA008C6 in its Precursor form, consumed the least amount of food when measured as the mean daily consumption and also as the total over the duration of the trial, compared with animals fed a standard rodent diet. These reductions in daily and total consumption were statistically significant ($p<0.05$). This would suggest that there might be palatability issues with this test preparation.

The consumption of the Ovine Glycoprotein MLA008C6 was also reduced but only at the high (1.0%w/w) dose. The animals fed the low dose (0.1% w/w) consumed slightly more when compared with those in the Control standard diet group. These differences for either experimental group were not statistically significant.

A comparison of the consumption of the MLA008C6 test preparation at 1.0% (w/w) and the Precursor form, also at 1%, showed that the differences were also not statistically significant. In all groups the weight gain by the animals was approximately the same with similar rates. Although the 1.0% MLA008C6 Precursor treated group displayed the least gain when compared with the animals on the Control standard diet, this difference, when expressed as the percentage total amount of weight gained over the trial, was not statistically significant. When compared to the Control animals fed a standard diet, the low dose Ovine Glcyoprotein MLA008C6 supplemented diet (0.1% w/w) produced a slight beneficial effect on the weight bearing during the acute and part of the chronic phases of the model. However, at the higher dose (1.0%) of this test preparation, there was no additional improvement in the weight bearing with values similar to or lower than those of the Control group. This might suggest that there is an inversely correlated dose response of this test preparation. In the animals fed the 1.0% Ovine Glycoprotein MLA008C6 Precursor supplemented diet, there was a clearly demonstrable beneficial effect on weight bearing. The values were above those of the Control group for much of the duration of the trial. Based on the weight bearing data, this Precursor form was notably more active in treating osteoarthritis than the other form.

The animals treated with Metacam at 5mg/kg body weight/day administered orally showed the expected improvement in the weight bearing throughout the trial except for an anomalous period from Day 39 onwards, where the values were actually below those of the Control group animals. However, the difference between the Control and the Metacam group was not statistically significant.

It is noteworthy that the Precursor form of the MLA008C6 Ovine Glycoprotein (at 1.0% of the diet) exhibited a similar degree of efficacy when compared with the weight bearing values of the Metacam treated animals, except for the acute phase where the latter was more effective. The expectations and the actual effects observed on the parameters monitored are summarised in Table 3.

Table 3. Key Experimental Findings from Testing Ovine Glycoprotein MLA008C6 at two Concentrations and Ovine Glycoprotein MLA008C6 Precursor for their Effects on the Modulation of Osteoarthritis in a Model Using Wistar Rats

Assay/Condition	Hypothesised Outcome	Result
Animal Study		
Food Consumption	All test animals would be expected to have a relatively similar daily intake of rodent chow.	The total and daily food consumption of rats fed the 1.0% Ovine Glycoprotein MLA008C6 Precursor was significantly reduced when compared with those on the control diet.
Body Weight Change	Young healthy animals involved in previous osteoarthritis trials have exhibited a steady weight gain.	The groups of rats fed the 1.0% Ovine Glycoprotein MLA008C6 and the 1.0% Ovine Glycoprotein MLA008C6 Precursor gained the least amount of weight when compared to those in the Control group.
Weight Bearing of the Osteoarthritic Knee Joint	Previous studies have shown that the MIA elicits a biphasic response in terms of the % weight bearing of the injected limb. In the acute, inflammatory phase then amount of weight placed on the MIA injected limb decreases to ~36% by Day 4 (post injection). This value improves before declining again to reach a similar value ~37 days after the MIA injection.	Of all the Experimental groups the group of rats fed the 1.0% Ovine Glycoprotein MLA008C6 Precursor exhibited the greatest efficacy in the improvement in the weight bearing of the osteoarthritic limb throughout most of the trial. This demonstrated efficacy was approximately the same as for Metacam in the Positive Control group which also showed an improvement in weight bearing throughout most of the trial. However, the differences in weight bearing of both groups were not statistically significant when compared to the rats in the Control group.

4.2 Presentation of Results:

In each of the graphs the values presented are the average or mean ± SD (standard deviation). Each of the graphs depicts data for the complete trial group (n=10).

4.2.1 Food Consumption and Animal Body Weights

The consumption of the various diets, which was available ad lib during the trial, is shown below in Figures 1 - 4. The average weight gain for each of the groups at the end of the trial and the changes in body weights during the trial are shown below in Figures 5 and 6.

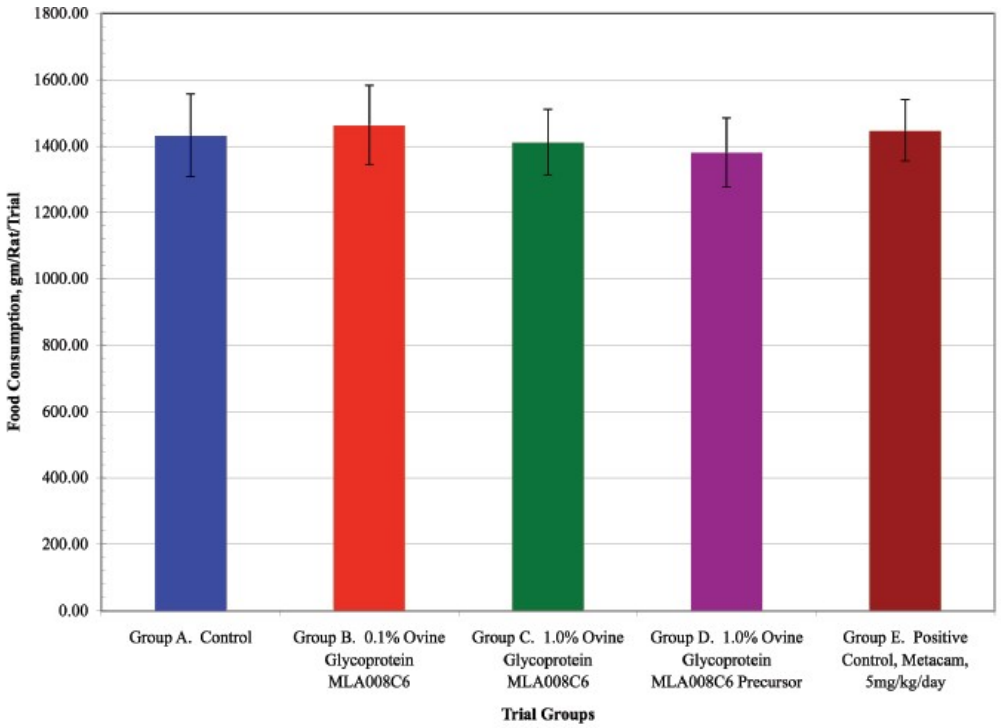

Figure 1. The total food consumption by each group of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10, except Group E, n=9). The data presented here are for the Control Standard Rodent Diet Group (Group A, blue bar); 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red bar); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green bar); 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple bar) and the Positive Control, Standard Rodent Diet + Metacam Group (Group E, brown bar).

Although there were differences in the total amount of food consumed by the various groups, when these totals were compared with the food consumption by the Control (Group A) animals, these differences were not significant, except for the Group D, 1.0% Ovine Glycoprotein MLA008C6 Precursor (See Table 4 below). The animals on the Control, Standard Rodent diet (Group A) consumed a total of 1431 gms over the duration of the trial. The animals on the 0.1% and 1.0% Ovine Glycoprotein MLA008C6 diets (Groups B and C) consumed 1462 and 1410gms respectively. The animals that received the 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D) consumed the least amount of food at 1380gms. This difference was significant at $p<0.05$. This could indicate that the MLA008C6 test compound is less palatable at the higher dose and that the Precursor form is even less palatable. The animals on the Standard Rodent diet plus Metacam (Positive Control, Group E) consumed 1446gms of food over the duration of the trial.

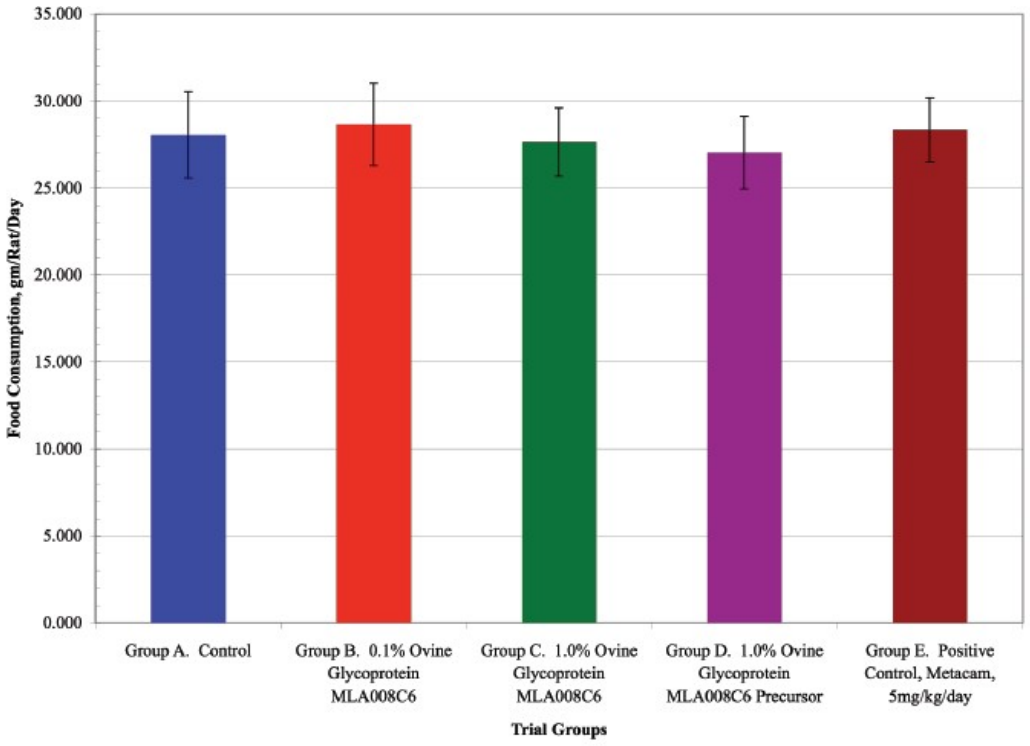

Figure 2. The daily food consumption by each group of rats for the trial. The values are the mean ± SD (Standard Deviation) for each group (n=10, except Group E, n=9). The data presented here are for the Control Standard Rodent Diet Group (Group A, blue bar); 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red bar); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green bar); 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple bar) and the Positive Control, Standard Rodent Diet + Metacam Group (Group E, brown bar).

The daily food consumption of all groups shows an identical pattern to the total food consumption as seen in Fig. 1 above. When compared with the Group A, Standard Rodent diet, the observed differences were not statistically significant, except for the comparison with the Group D, 1.0% Ovine Glycoprotein MLA008C6 Precursor. This difference was significant at $p<0.05$ (see Table 4 below).

IRL-P15-YOSA-1: Food & Test Preparation Consumption Statistics (Two Sample, Unpaired T-Test, Unequal Variance, Two Tailed)	
Total Food Consumption, (gm/Rat/Trial)	**p Values**
Group A (Standard Rodent Diet) vs Group B (0.1% Ovine Glycoprotein MLA008C6)	0.2078
Group A (Standard Rodent Diet) vs Group C (1.0% Ovine Glycoprotein MLA008C6)	0.3675
Group A (Standard Rodent Diet) vs Group D (1.0% Ovine Glycoprotein MLA008C6 Precursor)	0.0282
Group A (Standard Rodent Diet) vs Group E (Standard Rodent Diet + Metacam)	0.4910
Daily Food Consumption, (gm/Rat/Day)	**p Values**
Group A (Standard Rodent Diet) vs Group B (0.1% Ovine Glycoprotein MLA008C6)	0.2061
Group A (Standard Rodent Diet) vs Group C (1.0% Ovine Glycoprotein MLA008C6)	0.3671
Group A (Standard Rodent Diet) vs Group D (1.0% Ovine Glycoprotein MLA008C6 Precursor)	0.0290
Group A (Standard Rodent Diet) vs Group E (Standard Rodent Diet + Metacam)	0.4875
Total Test Preparation Consumption, (gm/Rat/Trial)	**p Values**
Group C (1.0% Ovine Glycoprotein MLA008C6) vs Group D (1.0% Ovine Glycoprotein MLA008C6 Precursor)	0.1322
Daily Test Preparation Consumption, (gm/Rat/Day)	**p Values**
Group C (1.0% Ovine Glycoprotein MLA008C6) vs Group D (1.0% Ovine Glycoprotein MLA008C6 Precursor)	0.0878
Statistically significant	$p<0.05$

Table 4. Consumption statistics of the total and daily food consumption as well as the total and daily consumption of the test preparations. The comparison is made between the Control group and the remaining four groups.

The total consumption of the test preparations by Groups B and C show that, as expected, the group receiving 1.0% of the MLA008C6 preparation (Group C) consumed approximately ten times as much of this as the Group B animals which were fed a diet supplemented with 0.1% MLA008C6 (Figure 3). The consumption of the 1.0% MLA008C6 Precursor preparation (Group D) was almost identical to that of the animals receiving the 1.0% MLA008C6 preparation (Figure 3).

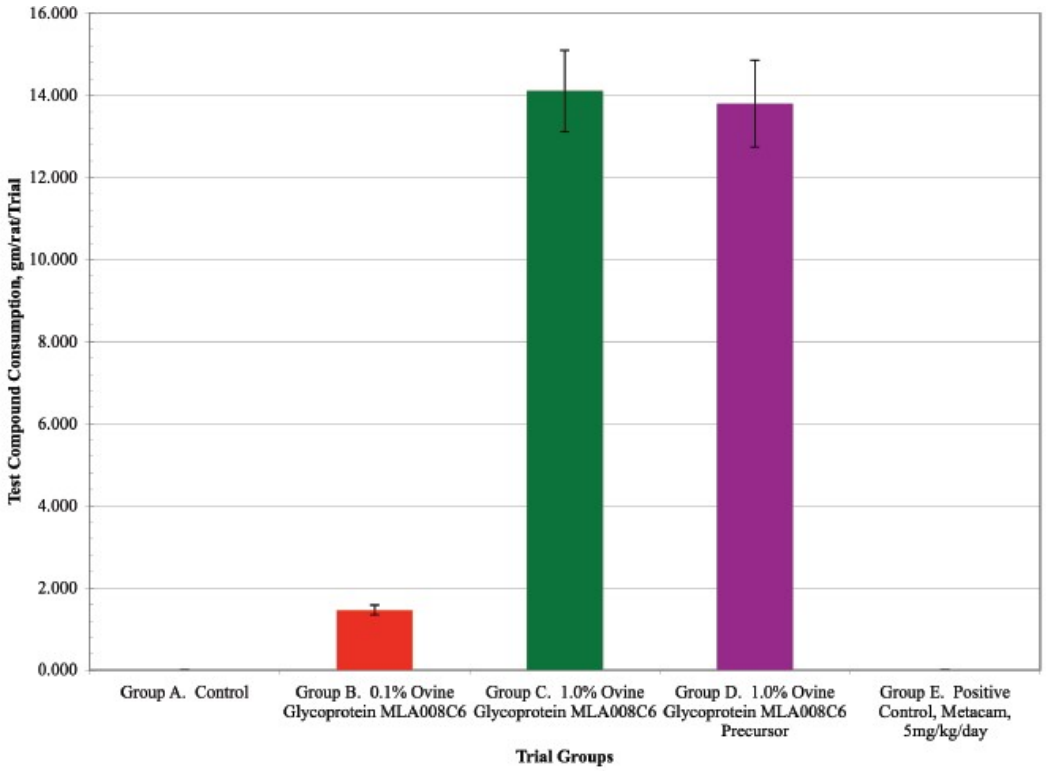

Figure 3. The total consumption of test preparations by the Experimental groups of rats. The values are the mean ± SD (Standard Deviation) for each group (n=10). The data presented here are for the 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red bar); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green bar); and the 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple bar).

The daily consumption of the test preparations by Groups B, C and D showed an identical pattern to that of the total test compound consumption (compare Figures 3 and 4). As shown in Table 4 above, a comparison of the total and daily amounts of the test preparations Ovine Glycoprotein MLA008C6 (Group C) and its Precursor form (Group D), demonstrates that that there were no statistically significant differences between the dosages of the two preparations.

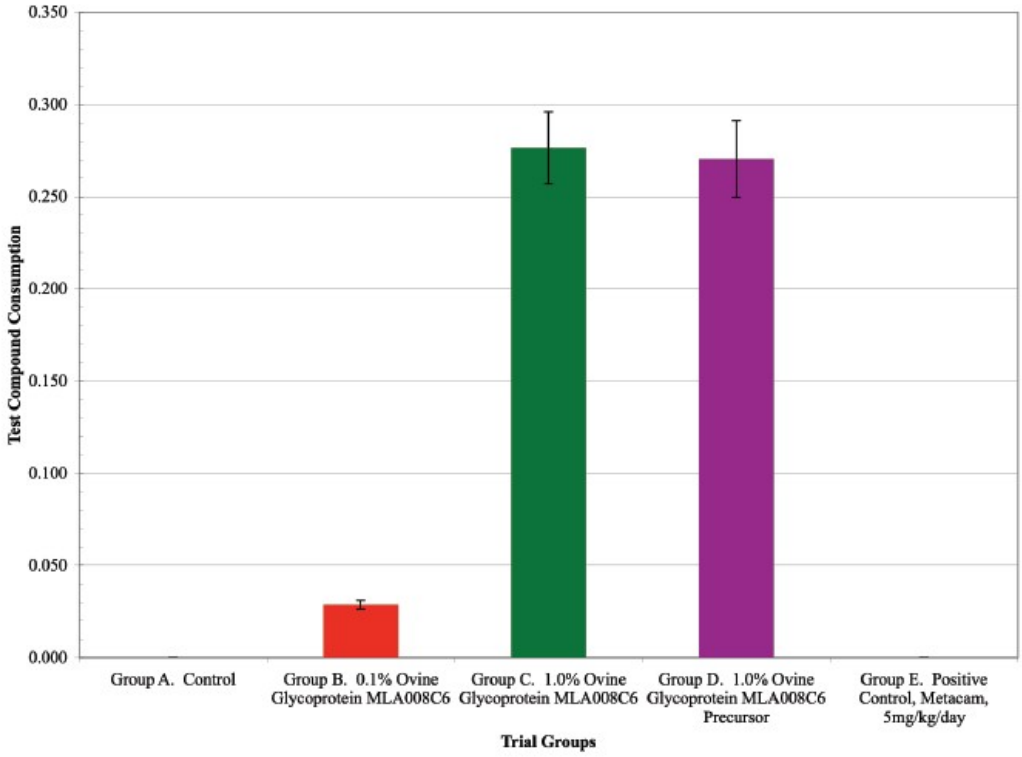

Figure 4. The daily test compound consumption for the Experimental groups of rats for the trial. The values are the mean ± SD (Standard Deviation) for each group (n=10). The data presented here are for the 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red bar); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green bar); and the 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple bar).

As the animals used in this study were young and consequently fast growing, it would be expected that the weight gain over the course of the trial would be substantial and this was what was observed. There were negligible differences in the gain in body weight of each of the five groups with the Standard Rodent Diet + Metacam (Group E) animals exhibiting the greatest gain at 317%, while the animals receiving the 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D), demonstrated the smallest gain at 281% (Figure 5). This latter finding was not unexpected as these animals consumed the least amount of food over the course of the trial (Figure 1). None of the differences in weight gain were statistically significant (See Table 5 in Appendix 2).

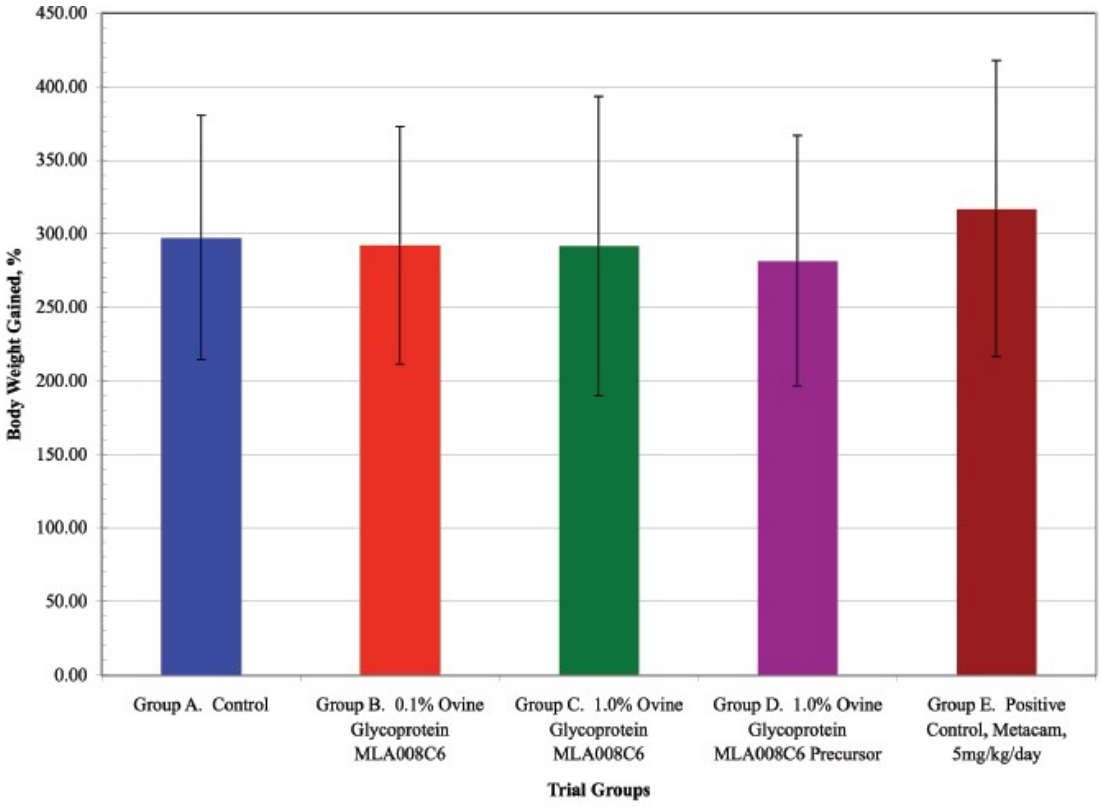

Figure 5. The weight gain by each group of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10, except Group E, n=9). The data presented here are for the Control Standard Rodent Diet Group (Group A, blue bar); 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red bar); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green bar); 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple bar) and the Positive Control, Standard Rodent Diet + Metacam Group (Group E, brown bar).

The animals from all five groups exhibited similar body weight increases over the course of the trial. At the time of MIA injection Day 22, they measured 278 to 304 gms increasing to between 416 to 442gms at the conclusion. The animals that received the 1.0% Ovine Glycoprotein MLA008C6 Precursor in their diet showed the smallest increase in weight (Figure 6). This finding is consistent with the food consumption and weight gain data as this group had the smallest food consumption.

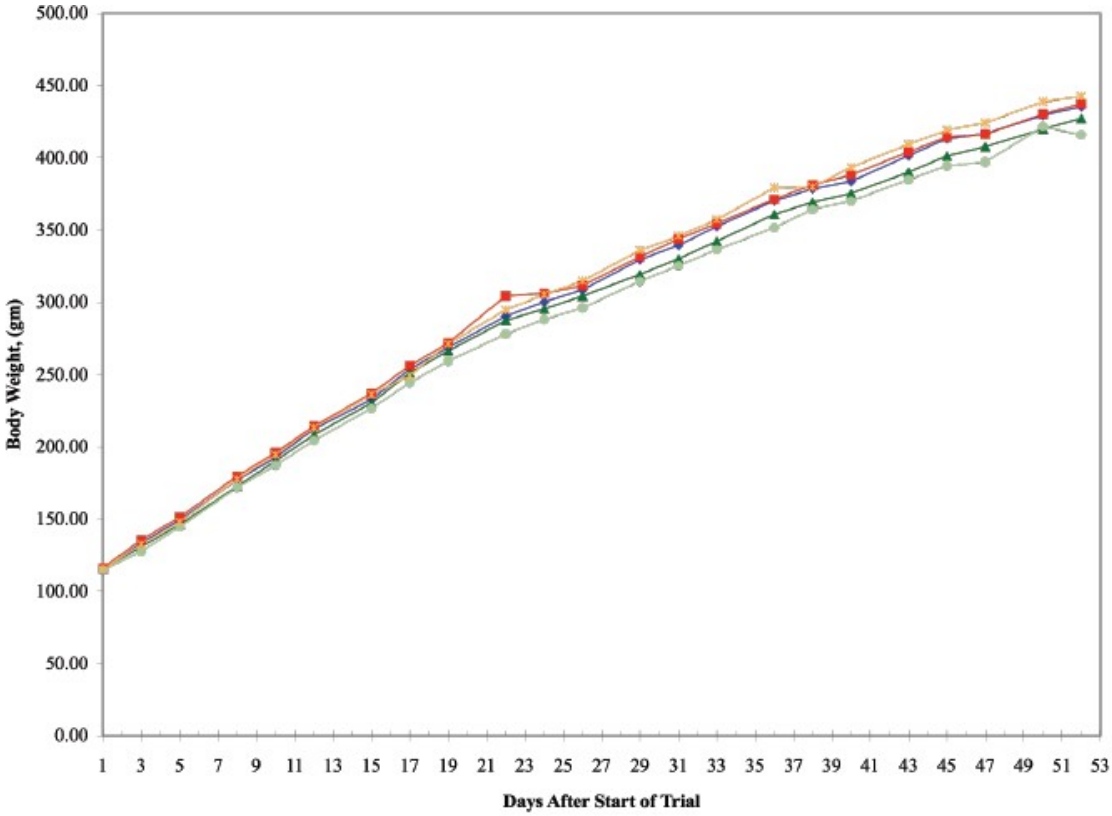

Figure 6. The body weight changes by each group of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10, except Group E, n=9). For the purposes of clarity the SD values have been omitted. The data presented here are from the Control Standard Rodent Diet Group (Group A, blue bar); 0.1% Ovine Glycoprotein MLA008C6 Group (Group B, red line); 1.0% Ovine Glycoprotein MLA008C6 Group (Group C, green line); 1.0% Ovine Glycoprotein MLA008C6 Precursor (Group D, purple line) and the Positive Control, Standard Rodent Diet + Metacam Group (Group E, brown line).

4.2.2 Right (Osteoarthritic) Hind Foot Weight Bearing Changes

Although rats are quadrapedal animals the incapacitance tester and its associated restrainer is designed to allow for the measurement of the amount of weight (gm) that the animal places on its left and right hind feet. A normal untreated rat would be expected to place 50% of its weight on each of its two hind feet. As osteoarthritis is a disease that clinically presents as pain (amongst other manifestations) in the affected joint, it would be expected that the degree of pain would correlate inversely with the amount of weight that the animal would place on the osteoarthritic (MIA injected) limb.

This measure of weight bearing is quantified as an index, which is the percentage of the weight bearing of the MIA injected limb divided by the combined weight bearing of both hind limbs. This formula (See Section 3.1.6, p8) allows for the normalization of animals of different body weights and therefore allows for an accurate comparison of the effect of the different treatments of the various groups over the course of the trial.

The comparisons of the percentage weight bearing on the right osteoarthritic limb for the Control, Standard Diet Group (A) with the two Experimental groups (B and C) fed diets supplemented

with 0.1 and 1.0% Ovine Glycoprotein MLA008C6 are presented in Figures 7 and 8. See Table 9 in Appendix 3 for the raw data).

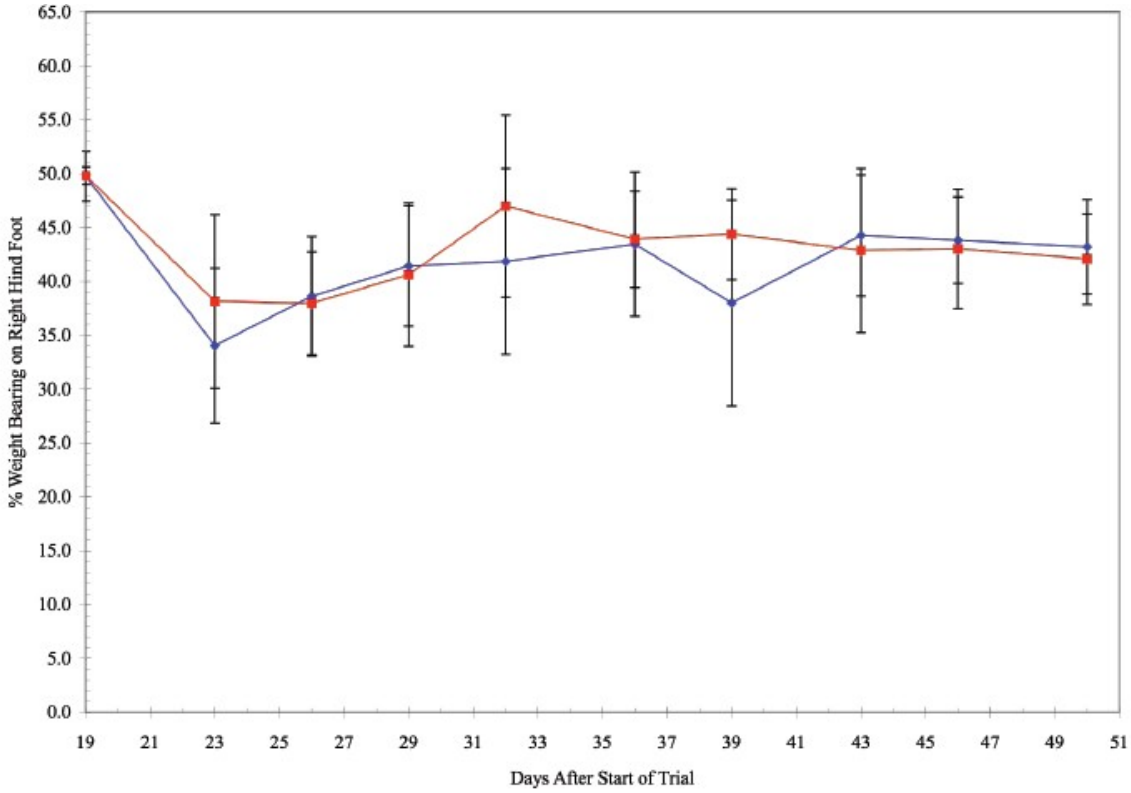

Figure 7. The percentage weight bearing on the right (osteoarthritic) hind foot for the Control and Experimental groups of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10). The data presented in this figure are for the Control, Standard Rodent Diet Group animals (Group A, blue line) and the 0.1% Ovine Glycoprotein MLA008C6 Group animals (Group B, red line).

As is typical of this model the animals in the Control group fed a Standard Rodent diet exhibited a biphasic response to the MIA injection. There was an initial inflammatory response which was maximal about two days after the injection at which point the knee joints were noticeably swollen and the mean weight bearing on the injected limb decreased to 34% (Figure 7). Over the next thirteen days the knee joint inflammation was gradually reduced and the weight bearing improved to 44% (Figure 7). Thereafter these animals exhibited a chronic relapse with the weight bearing decreasing to 38% by Day 39 (18 days post MIA injection). However, from Day 39 the weight bearing of this group sharply improved to a level of 44% by Day 43. This level was maintained through to the end of the study on Day 51 (Figure 7). It was unclear why this sudden improvement had occurred. In previous experiments the weight bearing for animals receiving a standard diet exhibited a consistent and progressive decline during the chronic phase of the model.

The animals fed a diet containing 0.1% Ovine Glycoprotein MLA008C6 exhibited a slight improvement in their weight bearing during the acute phase, reaching a level of 38% compared to 34% in the Controls (Figure 7). This might indicate that this dose of the glycoprotein has a slight but insignificant effect on this phase. But after Day 26 the values were similar to those of

the Control group animals, although there were indications of an improvement, albeit transient, in the weight bearing during the period from Day 32 to Day 43 (Figure 7).

Because this was a period of improvement in the weight bearing for the animals receiving the 0.1% Ovine Glycoprotein MLA008C6 there is an indication that this preparation actually does have an effect in reducing the cartilage degradation during this chronic phase. The reasons why the effect was not maintained are unclear but could be explained by the progressive severity of the disease in this model overcoming the beneficial effect afforded by this dose of the MLA008C6.

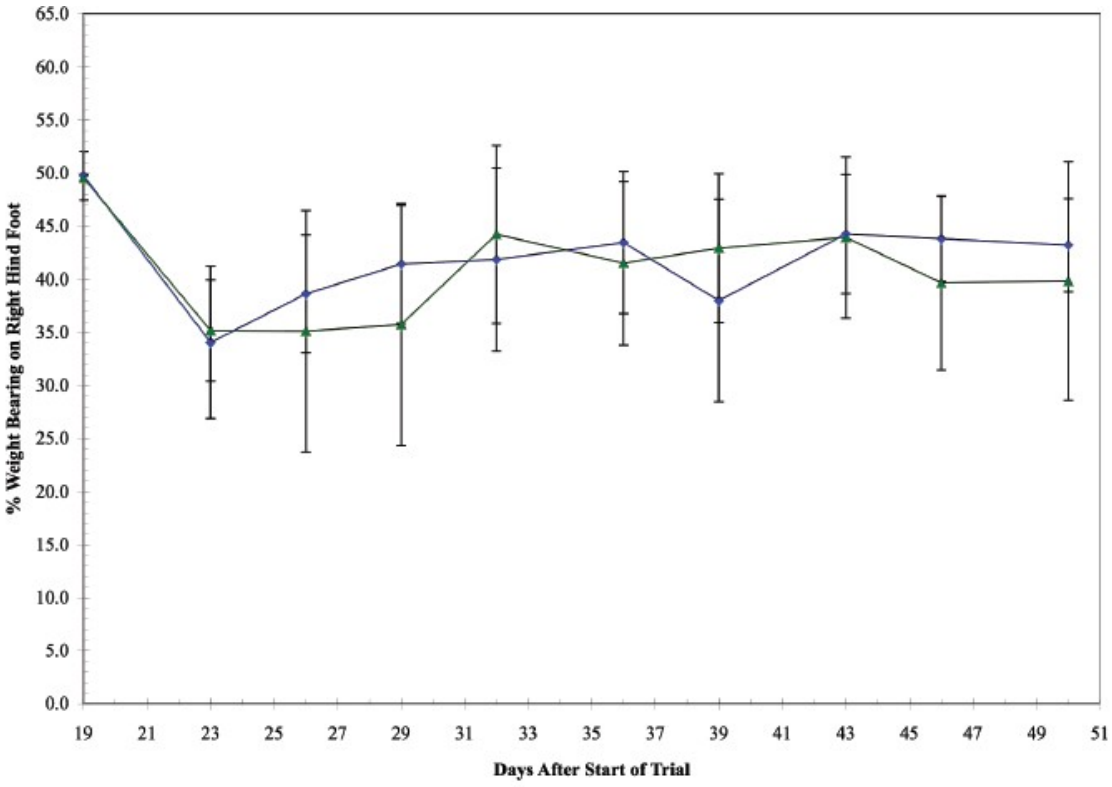

Figure 8. The percentage weight bearing on the right (osteoarthritic) hind foot for the Control and Experimental groups of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10). The data presented in this figure are for the Control, Standard Rodent Diet Group animals (Group A, blue line) and the 1.0% Ovine Glycoprotein MLA008C6 Group animals (Group C, green line).

Figure 8 shows that the animals fed a diet containing 1.0% Ovine Glycoprotein MLA008C6 did not, overall, exhibit any improvement in the weight bearing of the right osteoarthritic limb when compared with the animals on the standard diet. This is to be contrasted with the effect of the lower dose of the glycoprotein, which showed a possible positive effect on the acute phase of the disease and also during a subsequent part of the chronic phase. An inverse correlation between the dosage and the clinical efficacy might be indicated for this test preparation.

In Figure 9 the weight bearing data for the animals fed a diet containing 1.0% Ovine Glycoprotein MLA008C6 Precursor clearly demonstrates that this test preparation has had a beneficial effect on the weight bearing capacity of the MIA injected limb. The weight bearing values track above

those of the Control Group (A) animals for much of the trial from measurements in the acute phase through to Day 41 of the chronic phase, where the efficacy of the preparation appears to have worn off.

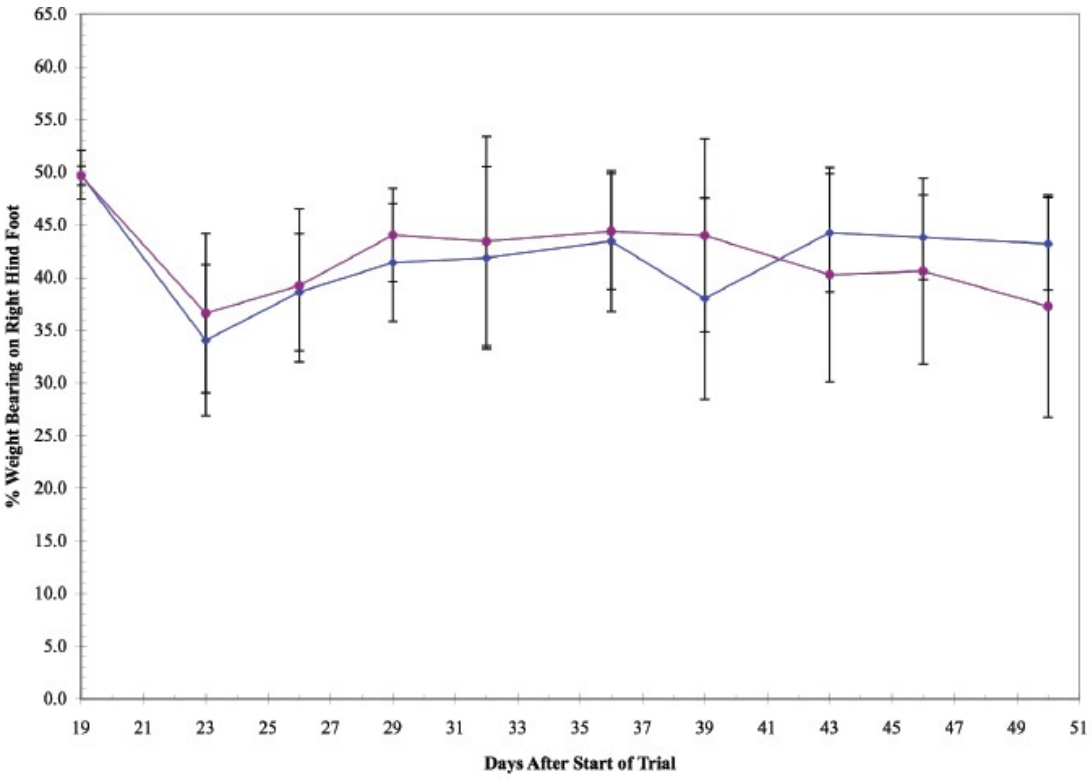

Figure 9. The percentage weight bearing on the right (osteoarthritic) hind foot for the Control and Experimental groups of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10). The data presented here are for the Control, Standard Rodent Diet Group animals (Group A, blue line) and the 1.0% Ovine Glycoprotein MLA008C6 Precursor Group animals (Group D, purple line).

Despite the animals in this group eating a significantly reduced amount of food, this test preparation was the most effective in affording an improved weight bearing capacity for a considerable proportion of the trial in both acute and chronic phases. However, the differences in weight bearing in this group compared to those of the Control group were not statistically significant (See Table 11 in Appendix 2).

The use of non-steroidal anti-inflammatory drugs to treat the pain aspect of osteoarthritis is well known, (McColl, G.J. (2001). Pharmacological therapies for the treatment of osteoarthritis. *Medical Journal of Australia 175: S108-S111*). The oral formulation of the NSAID Metacam has been administered by gavage in previous experiments employing this model. The effect has been to improve the weight bearing of the MIA injected limb during both the acute and chronic phases.

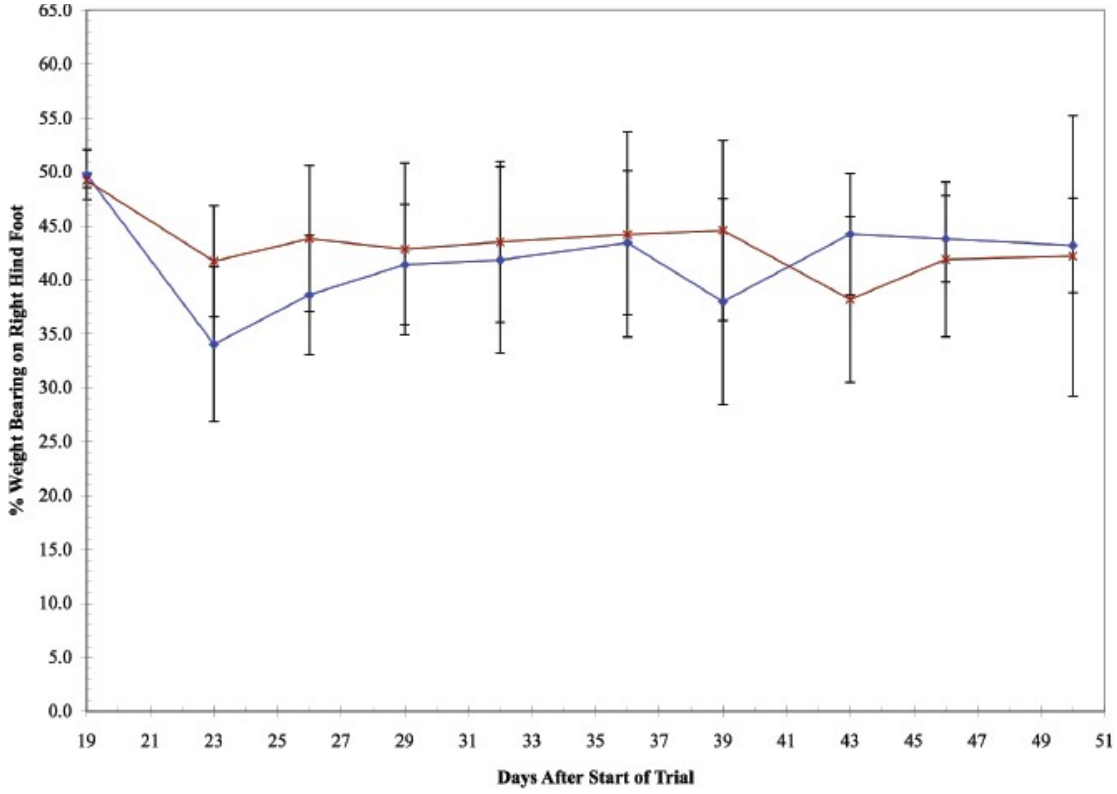

Figure 10. The percentage weight bearing on the right (osteoarthritic) hind foot for the Control and Experimental groups of rats over the trial period. The values are the mean ± SD (Standard Deviation) for each group (n=10, except Group E, n=9). The data presented here are for the Control, Standard Rodent Diet Group animals (Group A, blue line) and the Positive Control, Standard Diet + Metacam Group animals (Group E, brown line).

In this study it can be seen that the Metacam has exerted the expected effect with an improvement in the weight bearing values from the acute phase through to the chronic phase. In a similar pattern to the effect of 1.0% Ovine Glycoprotein MLA008C6 Precursor, the efficacy appears to cease on Day 41. It is difficult to reconcile the loss of efficacy of the Metacam at this point as the Control animals also exhibited an anomalous behaviour at the same time, as discussed for Figure 7.

A statistical analysis of the weight bearing data for the animals receiving Metacam showed that, when compared with the Control group, the differences were not statistically significant (See Table 11 in Appendix 2). Similarly to the effect of the 1.0% Ovine Glycoprotein MLA008C6 (Figure 9), this result was indicative of a trend.

There was no significant difference in the weight bearing ability for any of the experimental groups when compared with the control group using an ANOVA.

A nested ANOVA was also performed where the 5 groups were nested within two treatments (Control - which was only Group A and treatment which was the other 4). This was done for the whole dataset and the data up to Day 43. The ANOVA between groups until Day 39 was significant, however, only just, and when examined using the post-hoc Tukey's test it was between Groups 3 and 5, with the Metacam group being highest and the treatment group being the lowest. The ANOVA data for these comparisons is presented in Appendix 3.

5 Conclusions

1. The total and daily food consumption of the animals receiving the 1.0% Ovine Glycoprotein MLA008C6 was significantly reduced when compared to the animals receiving a standard diet. These animals also gained the least amount of weight.

2. The weight bearing data for the Control group animals fed a standard diet showed the expected reduction in both the acute and chronic phases of the model but to Day 39. From this point to the end of the trial on Day 51, these animals exhibited an anomalous and aberrant improvement in weight bearing for unknown reasons.

3. The 1.0% Ovine Glycoprotein MLA008C6 Precursor exhibited the greatest efficacy in improving the weight bearing capacity of the osteoarthritic limb of the animals fed a standard diet supplemented with this test preparation during the period from Day 19 to Day 39.

4. Similarly the animals fed a standard diet but treated with Metacam showed the expected improvement in weight bearing throughout the trial up to Day 39.

5. At the doses used in this experiment, the improvement in weight bearing of both the 1.0% Ovine Glycoprotein MLA008C6 Precursor and Metacam treated groups is indicative of a trend only, as the differences between these groups and the Control group animals were not statistically different when assessed using a T-Test at the individual timepoints or using an ANOVA evaluation.

6 Future Directions

We would recommend that the 1.0% Ovine Glycoprotein MLA008C6 Precursor be assessed further in this model using a dose response type experiment to determine the optimal concentration. It may be that the dosage and consumption in this study was sub-optimal and at the optimal concentration a statistically significant effect would be observed.

Study Sponsor

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Email: k.johnson@irl.cri.nz

Test Facilities and Test Site

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Email: pdavis@wnmeds.ac.nz

Dates

Starting date of experiments: 14/03/08
Completion date of experiments: 30/04/08

Statement

The study was not subject to quality assurance evaluation, but was conducted according to GLP guidelines.

Storage

Copies of the work order, labbook, the final report and all original raw data sheets associated with this study will remain in the archive room of Bioactivity Investigation Group for a period of not less than five years following the conclusion of this study. Records are maintained in a secure and protected area. Samples of test and reference items are kept in Fridge/freezer 5 at the Laboratory facilities (Room H5) at BIG, Wellington School of Medicine and Health Sciences, Wellington South.
Signed Date
Dr. Paul Davis
Study Director

7 Appendix 1. Detailed Study Protocols

The following is the study plan detailing the animal study and subsequent autopsy, histology, inflammatory and immune assays that were conducted. The study plan is a copy of that which was originally supplied to Lactopharma before commencement of the experiment. Any amendments to the study have been recorded at the end of the study plan.

UNIVERSITY of OTAGO

Te Whare Wānanga o Otāgo

STUDY PLAN for

INDUSTRIAL RESEARCH LTD

Title:

INVESTIGATION OF THE ANTI-INFLAMMATORY EFFECT OF MEAT DERIVED PEPTIDES USING THE IODOACETATE MODEL OF OSTEOARTHRITIS
(Project IRL-P15-YOSA-1)

(Version 5)

1. Objective

To determine the relative effectiveness of meat derived peptides at inhibiting joint degradation and inflammation in a model of osteoarthritis in rats. These efficacies are referenced to that produced by the anti-arthritic drug Meloxicam, which is administered to a group of rats immediately prior to the initiation of the arthritis. The arthritis model involves the injection of mono-sodium iodoacetate (MIA) into the knee joints of rats and subsequent observations on joint inflammation and weight bearing over the next 30 days.

2. Justification

Osteoarthritis (OA) is a disease of high economical and ethical importance. OA is a form of arthritis in which inflammation causes pain in the joints resulting from a variety of insults including age-related wear-and-tear, trauma, overweight, hereditary disorders, metabolic disorders or infection. In advanced stages, the patients suffer from severe pain and restriction of mobility. The consequence in many cases is an inability to work and often the substitution of the diseased joint with an artificial implant becomes inevitable. As cartilage tissue itself has only very limited capacities of self-renewing, the development of this disorder is chronic and progressive.

IRL has discovered several meat-derived peptides that have significant anti-inflammatory effects in a number of *in vitro* screens that measure different parameters and underlying mechanisms of inflammation. In particular some of these fractions have been shown to have an inhibitory effect on

the activated genes and enzymes that are associated with the degradation of the cartilaginous matrix of articular joints.

3. Test Items

Meat derived peptides (Supplied by the sponsor)
MLA008C6
MLA008C6 Pre-nutriceutical

4. Reference Item

Meloxicam (Boehringer Ingelheim, Lot No Y20801-D)

5. Study Sponsor

Industrial Research Ltd
P.O. Box 31-310
Lower Hutt

6. Contact:

Dr Keryn Johnson
Phone: (04)-931-3212
Fax: (04)-931-3055
Email: k.johnson@irl.cri.nz

7. Test Facilities and Test Sites

Bioactivity Investigation Group (BIG) and Biomedical Research Unit (BRU)
University of Otago, Wellington
PO Box 7343
Wellington South

8. Study Directors

Dr Paul Davis (Research Director)
Dr Nick Greenhill (Principal Investigator)
BIG
University of Otago, Wellington
PO Box 7343
Wellington South
Ph: (04)-918-6856

9. Dates

Date of approval of study plan:	May 2008
Starting date of experiments:	30th June 2008
Completion date of experiments:	21st August 2008

10. Test Methods

The methodology used is based on the procedure described by:

Bove *et al*, Weight bearing as a measure of disease progression and efficiency of anti-inflammatory compounds of monosodium iodoacetate-induced osteoarthritis. *Osteoarthritis and Cartilage (2003); 11: 821-830.*

11. Experimental Design

i) Justification for selection of test system

There are a number of *in vivo* models that are used for investigating osteoarthritis. The one to be used in this study involves the injection of iodoacetate into the knee joints of rats. This inhibits glyceraldehyde-3-phosphate dehydrogenase in chondrocytes of the cartilage resulting in the disruption of glycolysis and eventual cell death. This progressive loss of chondrocytes results in histologic and morphologic changes to the articular cartilage, closely resembling the changes seen in human osteoarthritis. It is ideal for testing agents that may preserve or repair cartilage structure. The iodoacetate suppresses the mobility, up-regulates some MMP activities and inhibits proteoglycan synthesis in the cartilage. Following the initial injection of iodoacetate there is an initial inflammatory response due to expansion of the synovial membrane by oedema fluid and fibrin with infiltration by macrophages, neutrophils and lymphocytes.

ii) **Characterisation of the Animals –**

Species and Strain: Wistar rats.

Sex: 10 male animals per group.

Age: (~4 weeks).

Weight Range: 52 - 78 grams at commencement (Mean ± 20%).

Diet: Standard rodent diet supplemented with the test compounds.

Drinking Water: Filtered tap water.

Housing and management: Room and boxes will be cleaned prior to start-up. The room will also be disinfected before commencement of the study. The boxes will be cleaned twice a week. The temperature of the room during the test will be maintained between 18 °C and 22°C. The temperature of the room during the test will be maintained between 18 and 22°C. Water and food will be checked three times a week. Illumination will involve a 12h light/dark cycle.

Source: Animals bred at the Hercus-Taieri Resource Unit, University of Otago, Dunedin.

Health status: Healthy.

Special requirements: None.

Medication history: None.

Animal Welfare: All animals will be handled similarly and with due regard for their welfare. Care of the animals will comply with the relevant Animal Welfare Act(s) and Regulations of New Zealand. The study design will be reviewed and approved by the Wellington School of Medicine Animal Ethics Committee prior to commencement.

The study plan is subject to amendments, variations and conditions that are required as part of the approval from the Wellington School of Medicine Animal Ethics Committee.

Euthanasia Endpoints: All animals will be euthanased at the end of the trial, 32 days after the administration of the iodoacetate. However, animals will also be euthanased if they: (1) lose 20% of their maximum weight; (2) lose 10% of their body weight over 24 hours; (3) are found moribund or with unalleviated pain; (4) exhibit self mutilation; (5) exhibit a severe clinical condition which warrants euthanasia, as determined by a veterinarian.

iii) Characterisation of the Test Systems

i) Monosodium Iodoacetate (MIA). Sigma Cat No. I-2512, Lot No 096K53081. Stored at -20 °C. Each animal will be given a single injection of (sterile filtered) 0.2µm monosodium iodoacetate (1mg in 25µl of saline) into the right knee joint. Each animal will also receive an injection of 25µl of sterile saline into the contralateral left knee joint.

ii) Meloxicam (Metacam). Each animal in the positive control group (Group D) will be given a daily dose of this drug at 5mg/kg body weight from the time of MIA administration onwards. The oral formulation of Metacam (15mg/ml) will be used and administered via oral gavage.

The volume to be delivered is calculated according to the following formula:

$$\text{Volume} = \frac{\text{Dose (mg/kg)} * \text{Body weight (kg)}}{\text{Solution concentration}}$$

iv) Diets:

Group A – Control group. Standard rodent diet (Poultry Research Unit, Massey University).

Group B – The standard rodent diet will be supplemented with MLA008C6 at a concentration of 0.1%. Prepared by Poultry Research Unit, Massey University.

Group C – The standard rodent diet will be supplemented with MLA008C6 at a concentration of 1.0%. Prepared by Poultry Research Unit, Massey University.

Group D – The standard rodent diet will be supplemented with MLA008C6 Pre-nutriceutical at a concentration of 1.0%. Prepared by Poultry Research Unit, Massey University.

Group E – Positive Control group. Standard rodent diet (Poultry Research Unit, Massey University), plus Metacam at 5mg/kg body weight.

v) Experimental Design

Animals will be randomly assigned to groups using body weights and Latin squaring to ensure that the mean body weights for each group are as close as possible.

All animals will be housed in boxes of two. Each box will be provided with pelleted supplemented food in wire hoppers and normal drinking water. The food consumption will be measured three times weekly and the hoppers topped up at those times. This will be continued until the conclusion of the experiment. There will be three weeks of feeding with the test supplements before the administration of the MIA.

The Meat Derived Peptide MLA008C6 will be tested at two doses of 0.1% & 1.0% diet, while the Meat Derived Peptide MLA008C6 Pre-nutriceutical will be tested at a single dose of 1.0% diet.

The experimental groups will be as follows:

Group A: Control group. 10 x male Wistar rats, aged ~4 weeks old with a body weight range of 52-78gm.

Group B: Meat Derived Peptide MLA008C6 at 0.1% (1gm/kg of food). 10 x male Wistar rats, aged ~4 weeks old with a body weight range of 52-78gm.

Group C: Meat Derived Peptide MLA008C6 at 1.0% (10gm/kg of food). 10 x male Wistar rats, aged ~4 weeks old with a body weight range of 52-78gm.

Group D: Meat Derived Peptide MLA008C6 Pre-nutriceutical at 1.0% (10gm/kg of food). 10 x male Wistar rats, aged ~4 weeks old with a body weight range of 52-78gm.

Group E: Positive Control group. Metacam at 5mg/kg. 10 x male Wistar rats, aged ~4 weeks old with a body weight range of 52-78gm.

During the preliminary period the rats will be acclimatised to the incapacitance tester (Linton Instrumentation, Norfolk, UK) in daily sessions prior to the administration of MIA. The animals will be housed in pairs to reduce isolation stress and individuals will be distinguished by tail marking with coloured crayon.

On Day 19 the incapacitance tester will be used to record baseline readings of the weight distribution for all animals on the day before the administration of the MIA. The animals will be placed in the angled Plexiglas chamber so that each hind foot is resting on a separate force plate. Once the animals are still, 5 consecutive readings of the weight of each hind foot will be recorded over a period of 5 to 10 seconds. The force exerted by each hind limb (measured in gms) will be averaged. Each animal's weight distribution will be recorded as the mean of the five readings.

The administration of the MIA will be staggered over two days (Days 22 and 23) with half of the animals from each group injected each of the two days.

The MIA (Sigma Cat. No I-2512, Lot No 096K53081) solution at 40mg/ml in sterile saline will be made fresh immediately prior to the MIA injections on both Days 22 and 23.

On either day 22 or day 23 the selected animals will be anaesthetized using Halothane (3.0%) and oxygen (2L/min.), administered initially in a purpose-made anaesthetic chamber and then on a nose cone connected to an anaesthetic machine with adequate ventilation and dispersal of gases.

Once the pedal reflexes of the hind feet have been abolished, a small area around each knee will be shaved. Using aseptic techniques, this area of the skin will be swabbed with a sterile swab/sponge soaked in a 0.5% Chlorhexidine solution in 70% Ethanol. The injection site will then be wiped with a dry sterile swab/sponge to remove any residual Chlorhexidine solution.

The MIA will be administered as a single 25µl injection through the infrapatellar ligament of the right knee using a 1ml syringe fitted with a 26 gauge sterile needle. The left contralateral knee will be injected similarly with 25µl of saline. A new syringe needle will be used for each knee and each animal. The animals will be closely monitored whilst recovering and can be ventilated during that time if necessary.

The animals in the positive control group will have Metacam administered daily by oral gavage from Day 22 onwards.

Body weights and food consumption will be measured three times weekly while cage side observations will be conducted daily using the procedures described in the monitoring sheet (Appendix A).

As the MIA administration will be staggered, then so will the measurement of the animals' weight distribution. This will enable measurements to be taken at the same time points following MIA administration for all animals. Thus one group will be measured on days 23, 26, 29, 32, 36, 39, 43, 46 and 50. The other group will be measured on days 24, 27, 30, 33, 37, 40, 44, 47 and 51. The change in hind foot weight distribution will be calculated by determining the difference in the weight (gm) between the left and right limbs.

Although the trial will end on Day 52, the removal of blood and tissues will be occur over Days 52 and 53. Each animal will be anaesthetized with Ketamine (100mg/kg) and Zylaxine (5mg/kg) and blood taken via cardiac puncture. The remaining blood will be dispensed into serum separating tubes and the serum dispensed into labelled cryovials and stored at -20^{0}C.

After exsanguination, the animals will be euthanased by cervical dislocation. The right knee joints from all animals will be excised and fixed in 4% buffered formalin. Further embedding, sectioning, histological or immunohistochemical assessment are not part of this study.

Serum Analyses

Characterisation of Test Systems

1. Rat IL1-β ELISA Kit (Rat specific). R&D Systems, Minneapolis, CatNo. RLB00. Stored at 4^{0}C.
2. Rat TNF-α ELISA Kit (Rat specific). R&D Systems, Minneapolis, CatNo. RTA00. Stored at 4^{0}C.

Experimental Analyses

1. Duplicate aliquots of each serum sample will be assayed for the cytokine IL1-β.
2. This will be performed according to the instructions in the manual that accompanies the ELISA kit for IL1-β.
3. Duplicate aliquots of each serum sample will be assayed for the cytokine TNF-α.
4. This will be performed according to the instructions in the manual that accompanies the ELISA kit for TNF-α.

Primary Endpoint: Change in joint capacitance measures between groups

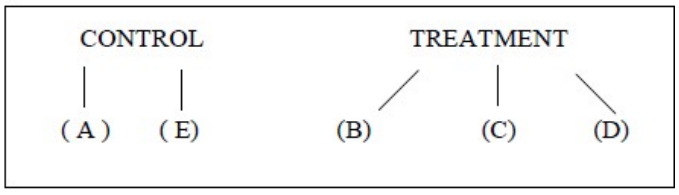
Secondary Endpoint: Change in animal welfare scores
Changes in the concentration of the cytokines in the serum samples

v) Analyses of Data

There will be five groups of 10 males each, with two serving as controls, and three being the experimental groups. A univariate 1-way nested ANOVA will be used to examine the differences between treatments and control and samples nested within treatments (see diagram below).

TREATMENTS
(fixed, orthogonal)

SAMPLES
(random, nested)



vi) Concurrent Medication-

In addition to the experimental treatments the only other drugs to be administered will be the Halothane for anaesthesia.

vii) Acceptability/Adverse Drug reactions (ADRS)

All adverse reactions that may be attributed to the administration of Test Substances will be documented and recorded.

viii) Deaths

The death of any animal during the course of the study will be recorded along with any relevant observations.

ix) Disposal of Animals

Animal carcasses and tissues not retained will be disposed according to the SOP on handling of biohazardous waste material.

x) Conduct of Study and Reporting Standards

The study will be conducted to the highest possible international research standards and practices. Unless otherwise specified, all procedures will follow what is stated in this study plan or according to Standard Operating Procedures (SOPs) that are contained in the SOP manuals of BIG and BRU.

A written report will be issued that describes the conduct of the study in detail and includes procedures actually used as well as any deviations to the plan that may have occurred. The report will include details of any observations and records made and any other circumstances that may affect the interpretation of results. A discussion of the study will also be included.

xi) Critical phases

The following are suggested as critical phases in the course of this study:
*Agreement on study protocol;
*Completion of the feeding phase prior to the initiation of the joint damage;
*Completion of the effect on the joint swelling;
*Completion of serum anlayses
*Submission of the final report.

xii) Observations

All observations and measurements will be recorded in the workbook (IRL-P15-YOSA-1: OA#7).

Statistical Methods

Descriptive statistics will be evaluated. Outlier removal will be determined using Cook's distances and z scores. Statistical analyses will be undertaken with and without outliers. Assumptions for normality will be tested using q-q plots of studentized residual values and assumptions for heterogeneity of variances will be tested using Leven's test and Residual plots. A model for the Univariate nested ANOVA will be custom made. SPSS 11 for Mac OSX will be used for assumption testing and statistical analysis. Graphs will display average percentage changes +/- SEM over time.

12. Records to be Maintained (as appropriate)

Workbook: IRL No
Excel worksheet: IRL-P15-YOSA-1.xls
SPSS data sheet: IRL-P15-YOSA-1.doc
Report in either Word or pdf. Printed and electronic versions of each will be prepared.

13. Quality Assurance Evaluation

The study described in this plan is not subject to quality assurance evaluation.

14. Study Plan Amendments and Deviations

Addenda and amendments to the Plan will be justified, signed and dated by the Study Director prior to implementation. All deviations to the plan will be recorded, signed and dated by the Research Director.

15. Ownership of Data, Confidentiality and Publication Rights

The data generated during this study will remain the property of the Sponsor. Confidentiality of study data and any information received from the Sponsor will be maintained during and after the study. Publication of material will remain at the sole discretion of the Sponsor.

16. Archives

Copies of all original raw data sheets associated with this study will remain in the archive room of Bioactivity Investigation Group for a period of not less than five years following the conclusion of this study. Records will be maintained in a secure and protected area.

Specimens will be moved to the storage facility designated by the Sponsor.

STUDY PLAN APPROVAL

Signed _____ Date _____

Dr. Nick Greenhill
Study Director

Signed _____ Date _____

Sponsor

Signed _____ Date _____

Dr. Paul Davis
Director, BIG

STUDY PLAN AMENDMENTS:

(Intended change to the study plan after the study initiation date – to be maintained with study plan)

Signed _____ Date _____

Dr. Paul Davis
Study Director

STUDY PLAN DEVIATIONS:

(Unintended departures of the study plan after the study initiation date – to be kept with raw data)

Description of Deviation:

Justification:

Signed _____ Date _____

Dr. Paul Davis
Study Director

Signed _____ Date _____

Dr Nicholas Greenhill
Principal Investigator

8 Appendix 2. Tabulated Data

The following is the tabulated data used to generate the graphs presented in the Results section.

Table 5. Data for total & daily food consumption as well as total and daily MLA008C6 & MLA008C6 Precursor consumption by each group of rats over the course of the trial. Data for Figures 1, 2, 3 and 4.

IRL-P15-YOSA-1: Food & MLA008C6/MLA008C6 Precursor Consumption								
Group	Mean Total Food Consumption Gm/Rat/Trial	S.D.	Mean Individual Food Consumption Gm/Rat/Day	S.D.	Mean Total Test Compound Consumption Gm/Rat/Trial	S.D.	Mean Individual Test Compound Consumption Gm/Rat/Day	S.D.
Group A. Control	1430.60	126.17	28.051	2.474	0.000	0.000	0.000	0.000
Group B. 0.1% Ovine Glycoprotein MLA008C6	1461.60	120.74	28.659	2.367	1.462	0.121	0.029	0.002
Group C. 1.0% Ovine Glycoprotein MLA008C6	1410.20	99.88	27.651	1.958	14.102	0.999	0.277	0.020
Group D. 1.0% Ovine Glycoprotein MLA008C6 Precursor	1379.30	105.57	27.045	2.070	13.793	1.056	0.270	0.021
Group E. Positive Control, Metacam, 5mg/kg/day	1445.80	93.47	28.349	1.833	0.000	0.000	0.000	0.000

Table 6. Data for the average percentage body weight gain by each group of rats over the course of the trial. Data for Figure 5.

IRL-P15-YOSA-1: Average Body Weight Gain (%)		
Group	Mean Body Weight Gain at End of Trial. N=10	S.D.
Group A. Control	297.04	83.44
Group B. 0.1% Ovine Glycoprotein MLA008C6	291.91	80.97
Group C. 1.0% Ovine Glycoprotein MLA008C6	291.39	101.92
Group D. 1.0% Ovine Glycoprotein MLA008C6 Precursor	281.12	85.53
Group E. Positive Control, Metacam, 5mg/kg/day	316.66	100.94

Table 7. Statistical analysis of the body weight gain (%) by each group of rats over the course of the trial.

IRL-P15-YOSA-1: % Body Weight Gain Statistics (Two Sample, Unpaired T-Test, Unequal Variance, Two Tailed)	
Mean Body Weight Gain at End of Trial	**p Values**
Group A (Standard Rodent Diet) vs Group B (0.1% Ovine Glycoprotein MLA008C6)	0.8906
Group A (Standard Rodent Diet) vs Group C (1.0% Ovine Glycoprotein MLA008C6)	0.8936
Group A (Standard Rodent Diet) vs Group D (1.0% Ovine Glycoprotein MLA008C6 Precursor)	0.6785
Group A (Standard Rodent Diet) vs Group E (Standard Rodent Diet + Metacam)	0.6414
Statistically significant	p<0.05

Table 8. Data for the body weight changes by each group of rats over the course of the trial. Data for Figure 6.

IRL-P15-YOSA-1. Body Weights.										
Day No	Group A. Control, Standard Diet. Body Weights. Mean. (N=10, 10 x Males)	S.D.	Group B. 0.1% Ovine Glycoprotein, MLA008C6. Body Weights. Mean. (N=10, 10 x Males)	S.D.	Group C. 1.0% Ovine Glycoprotein, MLA008C6. Body Weights. Mean. (N=10, 10 x Males)	S.D.	Group D. 1.0% Ovine Glycoprotein, MLA008C6 Precuror. Body Weights. Mean. (N=10, 10 x Males)	S.D.	Group E. Positive Control, Metacam, 5mg/kg/day. Body Weights. Mean. (N=10, 10 x Males)	S.D.
1	114.20	26.12	116.00	24.89	115.20	27.52	114.80	29.53	114.80	29.67
3	133.20	28.88	135.40	25.79	130.60	27.70	127.40	32.77	132.40	33.10
5	149.20	30.90	151.20	29.09	146.00	29.07	144.60	33.72	148.00	32.71
8	177.00	32.07	179.40	29.32	172.40	28.80	171.80	34.75	177.40	34.72
10	192.40	31.70	195.80	28.51	190.20	28.79	187.20	34.04	194.40	35.16
12	212.20	32.37	214.00	28.84	208.20	27.04	204.20	35.47	213.00	33.40
15	232.60	32.80	236.80	28.05	230.20	27.83	226.40	35.59	236.00	33.12
17	253.60	32.86	256.00	28.84	251.00	28.38	244.20	37.20	249.20	36.86
19	268.60	35.32	271.60	30.53	266.20	28.65	259.20	36.08	271.11	35.31
22	290.20	33.82	304.40	33.09	287.20	26.82	277.80	34.79	294.67	36.52
24	300.20	35.82	306.00	31.14	295.40	27.21	288.00	38.72	305.33	37.62
26	308.60	34.06	311.60	28.04	304.20	26.24	296.00	35.06	314.67	34.97
29	329.40	31.78	331.40	26.15	319.20	27.94	314.60	35.80	335.78	34.85
31	339.40	31.81	343.60	27.53	330.00	27.23	325.20	34.94	345.78	30.97
33	352.40	31.35	354.40	25.22	342.20	26.00	336.40	34.83	357.11	30.89
36	370.20	30.16	371.20	28.88	360.80	27.21	351.60	36.37	379.11	27.57
38	378.40	31.35	381.20	28.21	369.20	31.04	364.20	36.03	379.67	36.10
40	383.60	31.94	388.00	27.70	375.20	28.71	370.00	36.66	393.33	32.68
43	401.40	33.94	404.00	27.79	390.00	27.76	384.80	36.82	409.33	31.50
45	413.20	31.79	414.20	27.35	401.20	24.73	394.40	38.66	419.11	32.03
47	416.60	31.34	416.20	28.35	407.60	28.61	397.00	39.00	424.22	33.13
50	429.40	30.54	430.20	28.59	419.80	29.22	421.40	46.87	438.67	31.46
52	435.20	30.28	437.20	28.13	427.20	28.51	415.90	42.02	442.44	30.01

Table 9. Raw data for the right hind foot percentage weight bearing for each group of rats over the course of the trial. For brevity the means for each individual time point have been omitted. Note that Rat E8 died on 16/7/08, therefore n=9 for this group. Data from this table was used to create Table 10.

IRL-P15-YOSA-1: Inoapaoltanoe Test Raw Data										
Wt on Right Foot/(Wt on Right Foot + Wt on Left Foot) x 100										
Mean of 5 Individual Measurements										
	GROUP A		GROUP B		GROUP C		GROUP D		GROUP E	
DAY NO	RAT ID	ICP DATA	RAT ID	ICP DATA	RAT ID	ICP DATA	RAT ID	ICP DATA	RAT ID	ICP DATA
Day 19	A1	54.3	B1	48.7	C1	49.2	D1	49.5	E1	50.82
	A2	49.7	B2	49.5	C2	49.3	D2	48.1	E2	48.77
	A3	46.2	B3	50.7	C3	49.9	D3	51.4	E3	49.22
	A4	48.3	B4	50.9	C4	49.6	D4	49.9	E4	49.06
	A5	51.9	B5	50.0	C5	49.4	D5	49.8	E5	49.30
	A6	49.3	B6	49.5	C6	50.0	D6	49.2	E6	48.78
	A7	50.9	B7	50.3	C7	49.4	D7	49.5	E7	48.96
	A8	47.4	B8	50.5	C8	49.9	D8	50.6	E8	
	A9	50.5	B9	48.7	C9	49.5	D9	49.0	E9	48.74
	A10	49.3	B10	49.2	C10	49.2	D10	49.7	E10	49.45
Days 23 & 24	A1	29.1	B1	36.1	C1	34.4	D1	24.9	E1	35.72
	A2	22.2	B2	20.2	C2	33.2	D2	34.8	E2	35.79
	A3	36.2	B3	36.5	C3	33.7	D3	37.8	E3	43.39
	A4	30.4	B4	43.1	C4	32.6	D4	31.7	E4	39.07
	A5	42.5	B5	30.0	C5	36.6	D5	32.3	E5	45.65
	A6	33.5	B6	45.4	C6	34.6	D6	42.6	E6	45.89
	A7	39.9	B7	46.4	C7	39.9	D7	27.7	E7	50.48
	A8	25.2	B8	44.5	C8	43.4	D8	46.5	E8	
	A9	42.7	B9	39.4	C9	25.5	D9	42.5	E9	42.50
	A10	38.8	B10	39.9	C10	37.6	D10	45.9	E10	37.11
Days 26 & 27	A1	25.9	B1	33.8	C1	37.2	D1	25.5	E1	30.53
	A2	39.0	B2	30.7	C2	27.2	D2	46.1	E2	43.21
	A3	34.2	B3	42.1	C3	6.2	D3	41.4	E3	47.40
	A4	40.5	B4	41.7	C4	38.2	D4	37.5	E4	41.28
	A5	39.0	B5	45.8	C5	46.5	D5	33.6	E5	50.89
	A6	36.3	B6	35.9	C6	41.6	D6	43.9	E6	43.20
	A7	40.4	B7	38.3	C7	34.7	D7	46.4	E7	38.69
	A8	41.4	B8	34.3	C8	41.3	D8	48.4	E8	
	A9	44.9	B9	34.9	C9	36.3	D9	34.8	E9	33.27
	A10	44.7	B10	42.0	C10	41.8	D10	34.5	E10	45.99
Days 29 & 30	A1	33.1	B1	46.9	C1	5.5	D1	44.2	E1	30.00
	A2	41.8	B2	44.0	C2	38.3	D2	47.3	E2	49.31
	A3	47.4	B3	40.6	C3	39.5	D3	41.6	E3	46.88
	A4	41.6	B4	46.9	C4	35.0	D4	34.6	E4	37.55
	A5	41.5	B5	42.6	C5	44.0	D5	39.8	E5	33.07
	A6	47.6	B6	45.0	C6	32.8	D6	48.7	E6	33.87
	A7	45.3	B7	40.1	C7	47.2	D7	48.6	E7	50.24
	A8	30.5	B8	41.2	C8	36.0	D8	47.5	E8	
	A9	42.5	B9	25.3	C9	40.8	D9	44.6	E9	39.43
	A10	43.1	B10	33.6	C10	38.0	D10	43.4	E10	45.39
Days 32 & 33	A1	31.7	B1	46.2	C1	33.1	D1	27.1	E1	30.37
	A2	43.1	B2	45.6	C2	46.0	D2	47.1	E2	53.56
	A3	41.8	B3	41.9	C3	27.1	D3	41.9	E3	37.73
	A4	34.2	B4	37.3	C4	45.8	D4	34.4	E4	42.84
	A5	46.3	B5	47.3	C5	45.7	D5	39.9	E5	51.53
	A6	33.2	B6	30.1	C6	30.1	D6	35.9	E6	40.25
	A7	30.9	B7	37.6	C7	32.9	D7	39.3	E7	44.87
	A8	45.0	B8	61.4	C8	33.3	D8	48.9	E8	
	A9	26.4	B9	33.0	C9	41.4	D9	45.1	E9	40.16
	A10	48.1	B10	49.4	C10	47.0	D10	34.9	E10	50.46
Days 36 & 37	A1	30.2	B1	42.3	C1	28.8	D1	35.5	E1	24.44
	A2	37.4	B2	33.6	C2	44.7	D2	45.7	E2	45.19
	A3	40.9	B3	47.1	C3	30.1	D3	48.5	E3	49.41
	A4	42.9	B4	48.9	C4	46.3	D4	39.9	E4	49.91
	A5	43.6	B5	43.3	C5	42.1	D5	40.4	E5	38.37
	A6	45.0	B6	45.9	C6	48.8	D6	53.2	E6	40.54
	A7	43.0	B7	47.2	C7	52.2	D7	44.9	E7	44.94
	A8	45.8	B8	47.1	C8	44.6	D8	44.0	E8	
	A9	54.0	B9	41.2	C9	35.8	D9	40.5	E9	37.55
	A10	51.7	B10	42.4	C10	41.8	D10	51.2	E10	47.68
Days 39 & 40	A1	29.4	B1	39.8	C1	41.4	D1	32.7	E1	27.89
	A2	28.0	B2	42.9	C2	44.9	D2	36.6	E2	41.12
	A3	30.2	B3	52.5	C3	25.3	D3	44.0	E3	41.94
	A4	32.5	B4	46.8	C4	45.9	D4	35.4	E4	41.52
	A5	48.0	B5	45.0	C5	49.8	D5	38.4	E5	37.72
	A6	54.4	B6	42.0	C6	44.7	D6	53.2	E6	42.62
	A7	45.6	B7	42.4	C7	49.4	D7	33.7	E7	30.33
	A8	37.2	B8	44.0	C8	46.4	D8	41.1	E8	
	A9	39.3	B9	38.8	C9	39.2	D9	60.8	E9	48.59
	A10	40.3	B10	49.5	C10	42.2	D10	44.0	E10	49.40
Days 43 & 44	A1	31.2	B1	36.2	C1	32.8	D1	34.2	E1	22.44
	A2	44.0	B2	33.1	C2	44.9	D2	36.0	E2	38.47
	A3	43.6	B3	41.1	C3	47.1	D3	39.7	E3	39.59
	A4	45.7	B4	51.0	C4	48.0	D4	40.4	E4	37.08
	A5	50.4	B5	36.7	C5	51.6	D5	18.2	E5	47.36
	A6	45.7	B6	52.9	C6	48.7	D6	55.9	E6	37.70
	A7	46.5	B7	49.5	C7	48.2	D7	39.0	E7	38.80
	A8	38.8	B8	49.2	C8	36.2	D8	50.8	E8	
	A9	48.4	B9	33.7	C9	31.2	D9	43.9	E9	33.84
	A10	48.5	B10	45.3	C10	50.7	D10	44.5	E10	49.08
Days 46 & 47	A1	36.4	B1	46.6	C1	35.4	D1	28.5	E1	35.08
	A2	39.7	B2	41.8	C2	25.5	D2	38.8	E2	36.97
	A3	48.2	B3	41.7	C3	38.8	D3	45.1	E3	50.54
	A4	47.8	B4	52.5	C4	41.7	D4	33.0	E4	47.67
	A5	45.2	B5	39.3	C5	52.0	D5	30.3	E5	43.04
	A6	47.0	B6	46.3	C6	42.9	D6	43.1	E6	36.28
	A7	43.4	B7	37.3	C7	39.9	D7	39.0	E7	37.01
	A8	45.5	B8	37.1	C8	31.0	D8	45.4	E8	
	A9	39.5	B9	37.4	C9	37.7	D9	42.1	E9	36.59
	A10	45.3	B10	50.0	C10	51.7	D10	40.8	E10	34.11
Days 50 & 51	A1	43.2	B1	48.4	C1	20.1	D1	27.7	E1	16.17
	A2	37.9	B2	40.6	C2	19.2	D2	25.2	E2	45.47
	A3	38.4	B3	45.6	C3	43.6	D3	46.4	E3	50.10
	A4	38.6	B4	46.7	C4	46.4	D4	38.1	E4	47.81
	A5	42.5	B5	34.0	C5	46.5	D5	21.8	E5	51.73
	A6	47.3	B6	43.5	C6	50.3	D6	45.5	E6	31.26
	A7	46.5	B7	41.6	C7	43.6	D7	32.9	E7	31.91
	A8	51.6	B8	40.0	C8	36.9	D8	35.7	E8	
	A9	42.4	B9	41.4	C9	43.0	D9	40.7	E9	36.85
	A10	43.8	B10	38.9	C10	48.6	D10	38.6	E10	48.64

Table 10. Summarised data for the right hind foot percentage weight bearing for each group of rats over the course of the trial. Data for Figures 7, 8, 9 and 10.

IRL-P15-YOSA-1: % Weight Bearing on Right (Osteoarthritic) Hind Feet										
Day No	Group A. Control, Standard Diet. % Weight Bearing on Right Foot. Mean. (N=10, 10 x Males)	S.D.	Group B. 0.1% Ovine Glycoprotein, MLA008C6. % Weight Bearing on Right Foot. Mean. (N=10, 10 x Males)	S.D.	Group C. 1.0% Ovine Glycoprotein MLA008C6. % Weight Bearing on Right Foot. Mean. (N=10, 10 x Males)	S.D.	Group D. 1.0% Ovine Glycoprotein, MLA008C6 Precursor. % Weight Bearing on Right Foot. Mean. (N=10, 10 x Males)	S.D.	Group E. Positive Control, Metacam, 5mg/kg/day. % Weight Bearing on Right Foot. Mean. (N=10, 10 x Males)	S.D.
19	49.8	2.3	49.8	0.8	49.6	0.3	49.7	0.9	49.2	0.7
23	34.0	7.2	38.1	8.1	35.2	4.8	36.6	7.6	41.7	5.2
26	38.6	5.5	38.0	4.8	35.1	11.4	39.2	7.3	43.8	6.8
29	41.4	5.6	40.6	6.7	35.7	11.4	44.0	4.4	42.9	8.0
32	41.9	8.6	47.0	8.4	44.2	8.4	43.4	9.9	43.5	7.4
36	43.5	6.7	43.9	4.5	41.5	7.7	44.4	5.5	44.2	9.5
39	38.0	9.6	44.4	4.2	42.9	7.0	44.0	9.2	44.6	8.4
43	44.3	5.6	42.9	7.6	43.9	7.6	40.3	10.2	38.2	7.7
46	43.8	4.0	43.0	5.5	39.7	8.2	40.6	8.8	41.9	7.2
50	43.2	4.4	42.1	4.2	39.8	11.2	37.3	10.6	42.2	13.0

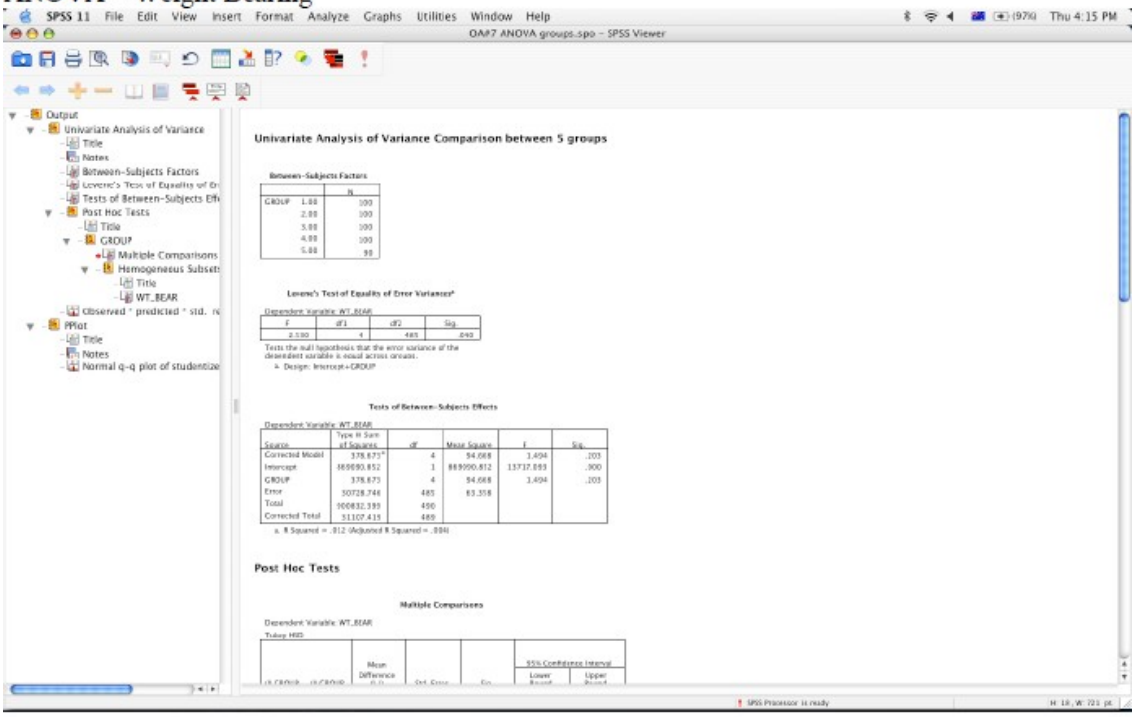
Table 11. Osteoarthritic limb weight bearing statistics. P-values were generated using the student t-test with a p- value of less than 0.05 indicative of statistical significance.

IRL-P15-YOSA-1. Incapacitance Weight Bearing Statistical Analysis (T-Test, Two Tailed, Unequal Variance, p Values). Comparison of Groups				
Day No	Control (Group A) vs 0.1% MLA CRY002 (Group B)	Control (Group A) vs 1.0% MLA CRY002 (Group C)	Control (Group A) vs 1.0% MLA CRY002 Precursor (Group D)	Control (Group A) vs Metacam5mg/kg/day (Group E)
19	0.9552	0.7781	0.8957	0.4935
23	0.2457	0.6856	0.4459	0.0155
26	0.7803	0.3964	0.8329	0.0875
29	0.7729	0.1792	0.2638	0.6598
32	0.1963	0.5428	0.7090	0.6572
36	0.8623	0.5549	0.7412	0.8429
39	0.0767	0.2069	0.1691	0.1270
43	0.6506	0.9170	0.2958	0.0714
46	0.7158	0.1747	0.3149	0.4978
50	0.5626	0.3918	0.1260	0.8305

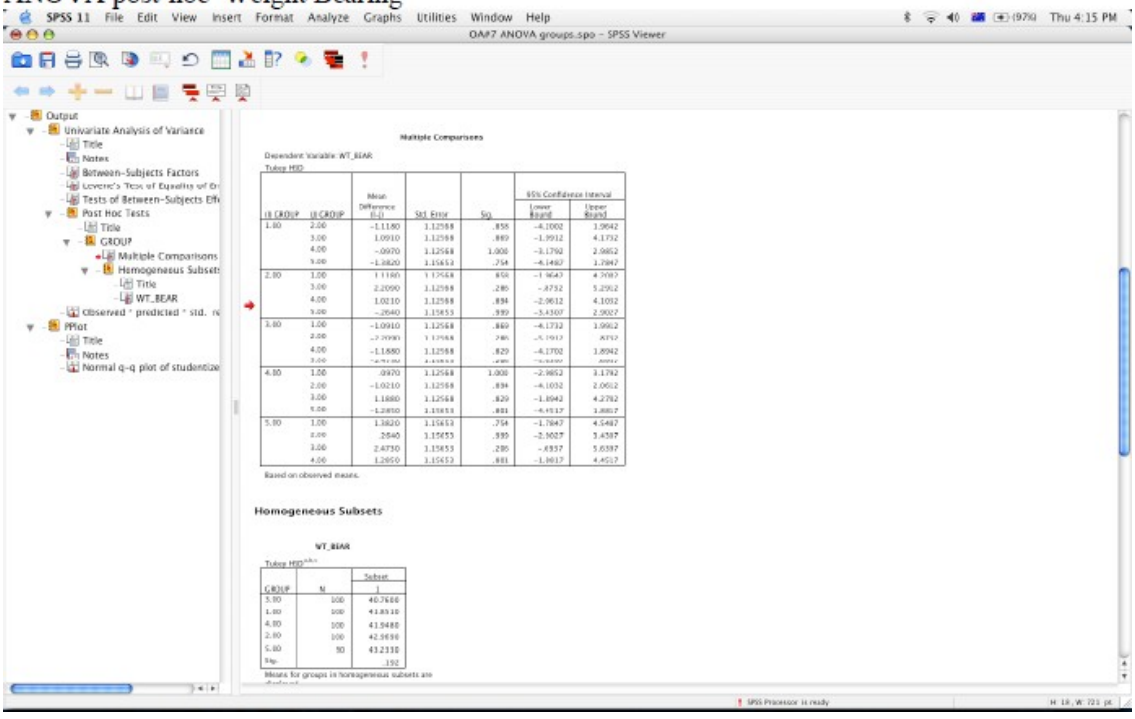
Appendix 3. Statistical Analyses

The statistical analyses are summarised in the following tables and charts.

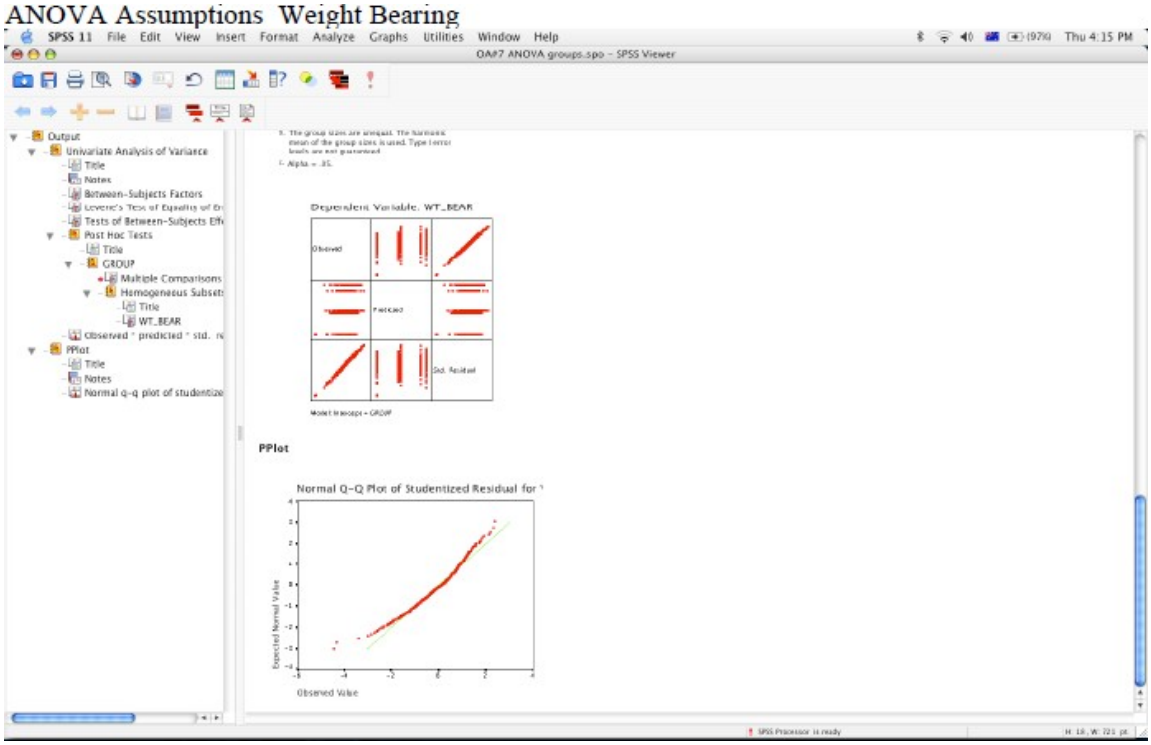
ANOVA – Weight Bearing



ANOVA post-hoc Weight Bearing



ANOVA Assumptions Weight Bearing



Nested ANOVA – Weight Bearing

Between-Subjects Factors

		Value Label	N
GROUP	1.00		100
	2.00		100
	3.00		100
	4.00		100
	5.00		90
TREATM	1.00	Control	100
	2.00	Treatment	390

Descriptive Statistics

Dependent Variable: WT_8EAR

GROUP	TREATM	Mean	Std. Deviation	N
1.00	Control	41.8510	7.25181	100
	Total	41.8510	7.25181	100
2.00	Treatment	42.9690	6.62358	100
	Total	42.9690	6.62358	100
3.00	Treatment	40.7600	9.20210	100
	Total	40.7600	9.20210	100
4.00	Treatment	41.9480	8.47620	100
	Total	41.9480	8.47620	100
5.00	Treatment	43.2330	7.99101	90
	Total	43.2330	7.99101	90
Total	Control	41.8510	7.25181	100
	Treatment	42.2017	8.15835	390
	Total	42.1301	7.97586	490

Levene's Test of Equality of Error Variances[a]

Dependent Variable: WT_8EAR

F	df1	df2	Sig.
2.530	4	485	.040

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+TREATM+GROUP(TREATM)

Tests of Between-Subjects Effects

Dependent Variable: WT_BEAR

Source		Type I Sum of Squares	df	Mean Square	F
Intercept	Hypothesis	869724.979	1	869724.979	7047.668
	Error	367.397	2.977	123.406[b]	
TREATM	Hypothesis	9.790	1	9.790	.079
	Error	364.690	2.935	124.242[c]	
GROUP(TREATM)	Hypothesis	368.883	3	122.961	1.941
	Error	30728.746	485	63.358[d]	

Tests of Between-Subjects Effects

Dependent Variable: WT_BEAR

Source		Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[a]
Intercept	Hypothesis	.000	1.000	7047.668	1.000
	Error				
TREATM	Hypothesis	.798	.026	.079	.055
	Error				
GROUP(TREATM)	Hypothesis	.122	.012	5.822	.501
	Error				

a. Computed using alpha = .05

b. 1.007 MS(GROUP(TREATM)) - 7.465E-03 MS(Error)

c. 1.021 MS(GROUP(TREATM)) - 2.148E-02 MS(Error)

d. MS(Error)

Expected Mean Squares[a,b]

Source	Variance Component		
	Var(GROUP(TREATM))	Var(Error)	Quadratic Term
Intercept	98.163	1.000	Intercept, TREATM
TREATM	99.529	1.000	TREATM
GROUP(TREATM)	97.436	1.000	
Error	.000	1.000	

a. For each source, the expected mean square equals to the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.

b. Expected Mean Squares are based on the Type I Sums of Squares.

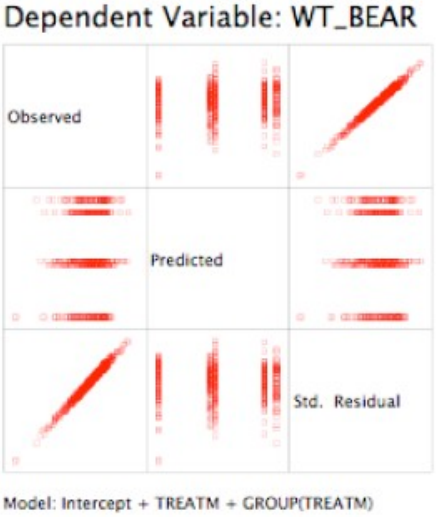
Estimated Marginal Means

Grand Mean

Dependent Variable: WT_BEAR

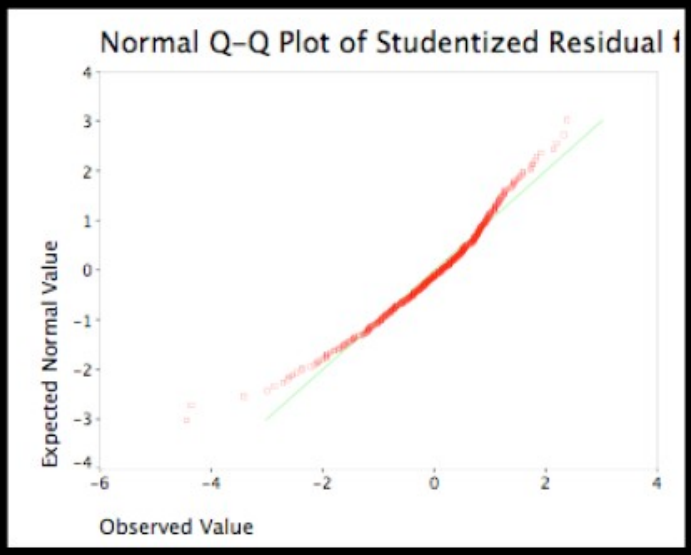
Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
42.152[a]	.360	41.445	42.859

a. Based on modified population marginal mean.



Model: Intercept + TREATM + GROUP(TREATM)

PPlot


Normal Q-Q Plot of Studentized Residual
Expected Normal Value
Observed Value
4
3
2
1
0
-1
-2
-3
-4
-6
-4
-2
0
2
4

ANOVA (until Day 39) Weight Bearing

Between-Subjects Factors

		N
GROUP	1.00	70
	2.00	70
	3.00	70
	4.00	70
	5.00	63

Levene's Test of Equality of Error Variances[a]

Dependent Variable: WT_BEAR

F	df1	df2	Sig.
1.242	4	338	.293

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.

a. Design: Intercept+GROUP

Tests of Between-Subjects Effects

Dependent Variable: WT_BEAR

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	647.228[a]	4	161.807	2.596	.036
Intercept	615942.101	1	615942.101	9880.831	.000
GROUP	647.228	4	161.807	2.596	.036
Error	21069.931	338	62.337		
Total	637643.504	343			
Corrected Total	21717.158	342			

a. R Squared = .030 (Adjusted R Squared = .018)

Post Hoc Tests

GROUP

Multiple Comparisons

Dependent Variable: WT_8EAR

Tukey HSD

(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-2.0771	1.33456	.527	-5.7373	1.5830
	3.00	.4329	1.33456	.998	-3.2273	4.0930
	4.00	-2.0200	1.33456	.554	-5.6801	1.6401
	5.00	-3.2554	1.37113	.125	-7.0158	.5050
2.00	1.00	2.0771	1.33456	.527	-1.5830	5.7373
	3.00	2.5100	1.33456	.330	-1.1501	6.1701
	4.00	.0571	1.33456	1.000	-3.6030	3.7173
	5.00	-1.1783	1.37113	.911	-4.9387	2.5822
3.00	1.00	-.4329	1.33456	.998	-4.0930	3.2273
	2.00	-2.5100	1.33456	.330	-6.1701	1.1501
	4.00	-2.4529	1.33456	.353	-6.1130	1.2073
	5.00	-3.6883	1.37113	.058	-7.4487	.0722
4.00	1.00	2.0200	1.33456	.554	-1.6401	5.6801
	2.00	-.0571	1.33456	1.000	-3.7173	3.6030
	3.00	2.4529	1.33456	.353	-1.2073	6.1130
	5.00	-1.2354	1.37113	.896	-4.9958	2.5250
5.00	1.00	3.2554	1.37113	.125	-.5050	7.0158
	2.00	1.1783	1.37113	.911	-2.5822	4.9387
	3.00	3.6883	1.37113	.058	-.0722	7.4487
	4.00	1.2354	1.37113	.896	-2.5250	4.9958

Based on observed means.

Homogeneous Subsets

WT_BEAR

Tukey HSD[a,b,c]

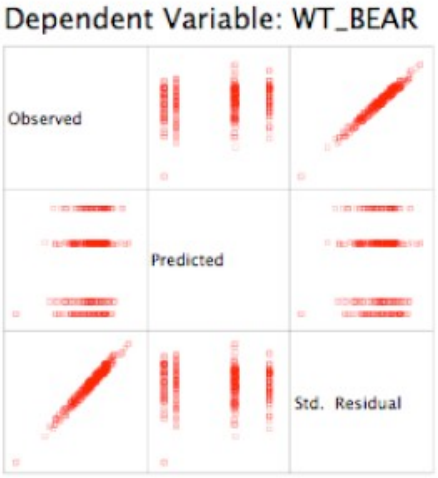
GROUP	N	Subset 1
3.00	70	40.5971
1.00	70	41.0300
4.00	70	43.0500
2.00	70	43.1071
5.00	63	44.2854
Sig.		.051

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = 62.337.

a. Uses Harmonic Mean Sample Size = 68.478.

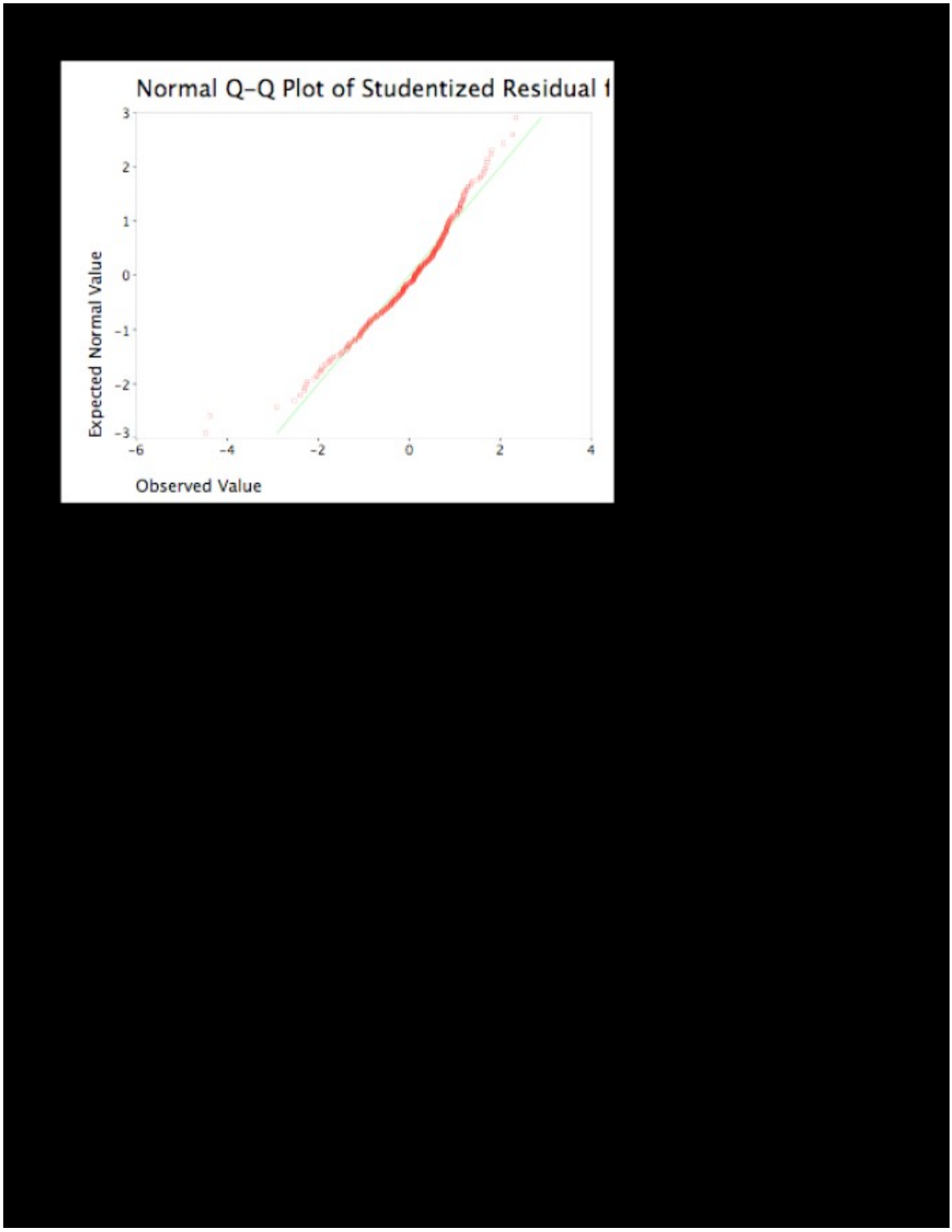
b. The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

c. Alpha = .05.

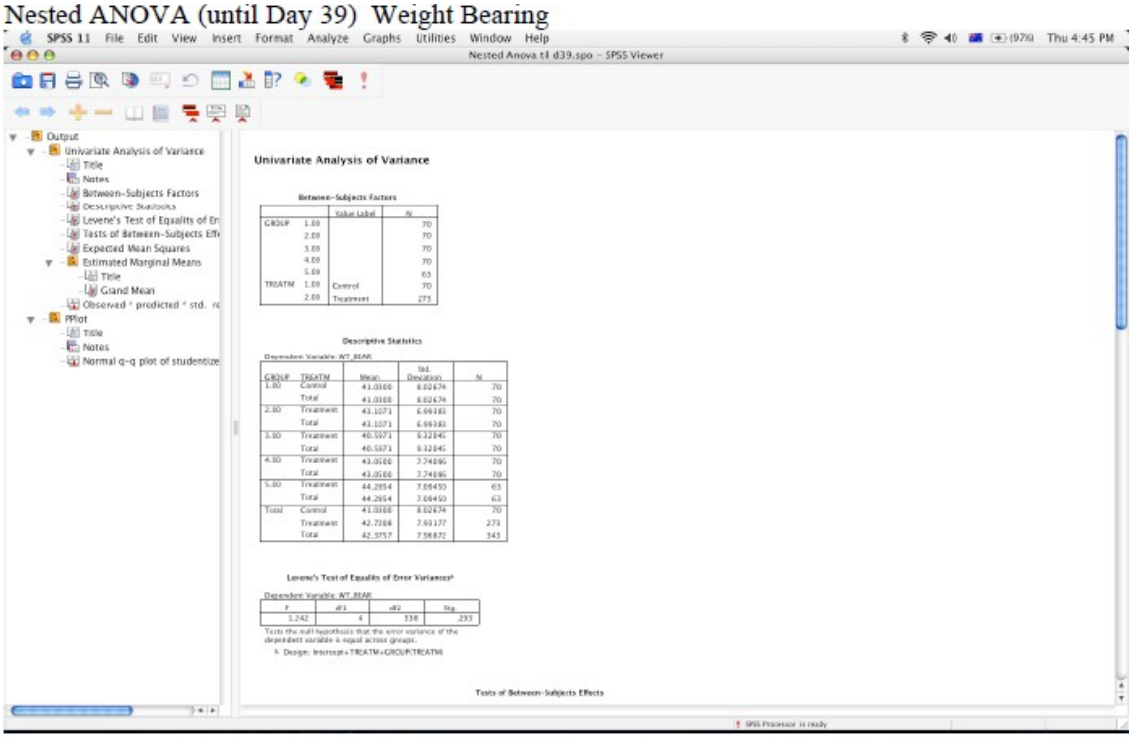


Model: Intercept + GROUP

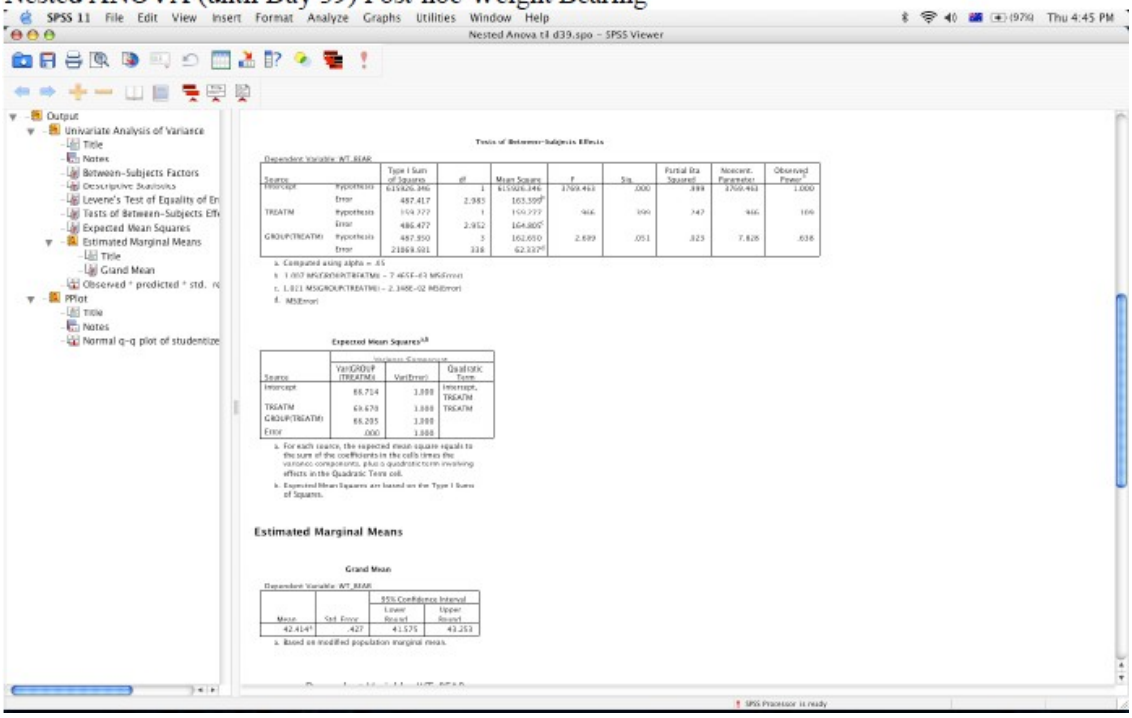
PPlot


Normal Q-Q Plot of Studentized Residual
Expected Normal Value
Observed Value
3
2
1
0
-1
-2
-3
-6
-4
-2
0
2
4

Nested ANOVA (until Day 39) Weight Bearing



Nested ANOVA (until Day 39) Post-hoc Weight Bearing



Nested ANOVA (until Day 39) Assumptions Weight Bearing