

Chapter 3

Changes in body composition of mature Coopworth rams and ewes during periods of feed restriction and realimentation.

3.1 Introduction

A fluctuating feed supply is part of most grazing systems. Fluctuations in feed intake will often induce periods of either weight gain, weight maintenance or weight loss for the grazing animal. The losses in production which are associated with the periods of weight stasis, or weight loss are sometimes partially, or fully compensated for by an acceleration of growth when feed supplies become non-limiting. The focus of research on body weight changes, induced by differences in feeding levels, has been on the phenomena of compensatory growth (for reviews see O' Donovan, 1984; Ryan, 1990; Carstens, 1995). Body compositional changes during the phase of compensatory growth have been well documented in sheep (Graham and Searle, 1975; Ledin, 1983; Butler Hogg, 1984). In contrast, compositional changes during the period of weight loss have received less attention, which is unfortunate given that Butler-Hogg (1984) indicated that the composition of an animal at the end of a period of nutritional restriction has an effect on the subsequent changes in body composition during compensatory growth. More precise knowledge of how individual animals react to a period of weight loss could provide the key to understanding relationships between compensatory growth, maintenance efficiency and body component changes during both feed restriction and the subsequent realimentation phase.

Studies that have documented body compositional changes during weight loss in immature sheep indicate that over time there are distinct priorities for tissue depletion in order to meet the energy requirements of the nutritional deficit. The early phase of weight loss is characterised by a loss of lean tissue, initially from the visceral organs and then lean from the carcass muscles, with little or no loss of fat (Drew and Reid, 1975a; Searle *et al.*, 1979; Thorton, *et al.*, 1979; Aziz *et al.*, 1992). As the severity of the weight loss increases, the rate at which fat is mobilised increases and the rate at which lean is mobilised

decreases. Little and Sandland (1975), indicated that the most labile fat reserve was the subcutaneous depot during a weight loss phase with little loss of tissue from the internal fat depots. It is interesting to note that the work of Fattat *et al.* (1984), Drouillard *et al.* (1991a) and recently Chowdury *et al.* (1995) indicated that if protein is sufficient in the diet during the weight loss phase, fat was mobilised to support protein conservation and even protein gain in immature animals.

In mature sheep, it appears as though fat is preferentially mobilised whilst carcass lean is conserved (Thorton *et al.*, 1979; Butler-Hogg, 1984). Keenan and McManus (1969) showed that 39% of initial fat reserves were lost whilst only 13% of initial protein reserves were lost during a period of four weeks where the sheep were fed at 30% of maintenance. Burton *et al.* (1974) showed that the relative rate (allometric coefficients) of fat mobilisation with respect to EBW was 1.9 whilst those for protein and water were low at 0.5 and 0.4 respectively. In contrast, Panaretou (1964) and Hight and Barton (1965) both showed that in mature ewes carcass fat depots remained constant despite severe levels of weight loss.

The majority of the studies that have examined tissue loss during weight loss in sheep have used either females or castrates. These studies show that generally sheep of these sexes will attempt to conserve fat tissue in preference to carcass lean (Winter, 1971; Searle *et al.*, 1979; Aziz *et al.*, 1992). In contrast tissue mobilisation of intact males during weight loss in sheep has received far less attention. Kabbali *et al.* (1992b) showed that in immature rams there was a preferential loss from the visceral organs and internal fat in the early stages of weight loss, with losses in the later phase primarily occurring in the carcass. During re-feeding, there was a priority for replenishment of the visceral organs and less for fat generation resulting in leaner carcasses. Notter *et al.* (1983) noted that rams fed at maintenance increased the proportion of body fat, which has also been shown by work of Vangen and Thompson (1992). Price (1975) compared bulls and steers and found that the weight loss in entires was far higher than in castrates and attributed the difference to steers initially having higher body energy levels (higher fat). He suggested that the steers had a greater proportion of fat in the body and therefore were able to obtain a greater proportion

of the energy deficit from this depot. Steen (1995), also showed that, in cattle, a period of restricted feeding had a greater effect on bulls when compared to steers and heifers and that this was a result of the higher proportion of lean in the bulls.

Recent studies on fallow deer by Jopson, *et al.* (1996) provide interesting results for entire males compared to castrates during weight loss situations. Body compositional changes in intact and castrated males were monitored during a natural period (the rut) and an enforced period of feed restriction. They showed that functional males were able to conserve carcass lean in preference to fat, whilst castrates and effective castrates (ie. intact males outside of the rut period) are unable to preferentially conserve lean relative to fat. Jopson *et al.* (1996) concluded that lean conservation occurs when testosterone or one of its derivatives is present at high levels in the intact male.

There appears to be no comparisons of weight loss in mature males and females under the same experimental conditions. Despite this, the evidence from studies on deer leads to the hypothesis that under a period of weight loss the female, because of a fat based metabolism, will conserve fat tissue, whilst the growth based metabolism of the male results in a priority for lean tissue conservation (Fennessy *et al.*, 1991).

The models proposed by Black (1974), indicated that the rate at which weight was lost would have an effect on the relative proportions of tissues that were lost during weight loss periods. The work of Searle and Graham, (1972) and Butler-Hogg (1984), confirmed that in sheep undergoing rapid weight loss a greater absolute weight of protein is lost whilst for sheep that have a lower rate of weight loss a greater absolute weight of fat is lost. It is interesting to speculate on whether the rate of weight loss will interact with either sex or initial body reserves and whether these effects will affect the responses that occur during realimentation. Although sex has been shown to have an effect on body composition during weight loss, no experiments which examined the tissues deposited during the realimentation phase have found an effect of sex.

Changes in body composition during the realimentation phase (compensatory phase) in sheep appear to be the reverse of the patterns of tissue depletion during weight loss. Drew and Reid (1975a) and Ryan *et al.* (1993b), showed that the replenishment of visceral organs is an initial priority in the realimentation phase, which accounts for the majority of the increase in protein deposition that has been noted in most experiments that have studied body composition during compensatory growth in sheep. Fat deposition increases in the latter stages of realimentation (Searle *et al.*, 1979; Ryan *et al.*, 1993b) such that there is no difference between restricted and normally grown sheep after sufficient time of realimentation (Butler-Hogg, 1984; Ryan *et al.*, 1993b).

In mature sheep, Keenan *et al.* (1969) and Burton *et al.* (1974) showed that the preferential deposition of lean tissue that occurred during realimentation, continued until the weight prior to the weight loss was reached. The relative rates of tissue growth with respect to EBW in mature sheep that were undergoing realimentation for fat (1.17) and protein (0.86) reported by Burton *et al.* (1974), when compared to those for weight loss, provides evidence for a greater priority for lean deposition after a period of weight loss. However, there is evidence that suggests that the compensatory growth response noted in relatively immature sheep may not occur in mature sheep (Hight and Barton, 1965; Butler-Hogg and Tulloh, 1982).

One of the mechanisms that has been identified as contributing to compensatory growth is that of a decline in maintenance requirements after the period of weight loss. However in most studies involving compensatory growth in sheep, *ad libitum* feeding has occurred during the period of realimentation, such that it remains unclear whether the change in the metabolic pathways that occurs during the weight loss phase (Greeff *et al.*, 1986b) will have an effect on the priorities for feed utilisation during the realimentation phase

The level of fat in the body may have an impact on the animals' ability to cope with a phase of nutritional stress and alter the response of the animal during the realimentation period. The use of the lines selected for a divergence in backfat thickness

provided the opportunity to examine the effects of prior body composition on responses during both a restriction and realimentation phase.

3.1.1 Null Hypotheses

- 1) *That there was no difference in the relative priorities and timing for tissue depletion in rams and ewes that are subjected to the same level of weight loss at maturity.*
- 2) *There was no effect of initial fat reserves of sheep on the priorities for tissue depletion during weight loss.*
- 3) *That the rate of weight loss resulting from a feed restriction had no effect on the timing and priorities for tissue loss during weight loss.*
- 4) *That a period of weight loss will not effect the utilisation of feed for body composition maintenance and growth in sheep during a period of realimentation.*

3.2 Materials and methods

This experiment was conducted with approval from the University of New England Animal Welfare Committee (PACEC 930057, 930058, 930105 and 940011).

3.2.1 Animals

The sheep used in this experiment were from the Coopworth high backfat and control selection lines developed at Invermay AgResearch New Zealand (Fennessy *et al.*, 1987). The high backfat Coopworth line was the result of a divergent selection for ultrasonic backfat depth at the 12th rib (C) site adjusted for live weight at 6 and 8 months respectively for rams and ewes. The randomly selected flock (control) had also been maintained during this period. In 1988, selection emphasis changed to include backfat measurements at both the C and GR (ultrasonic tissue depth measured at the 13th rib, 110 mm from the midline) sites in BLUP procedures for genetic evaluation (Fennessy *et al.*, 1989). Embryo transplant programmes were conducted in both 1989 and 1990 (Afonso, 1992). Embryos were collected from the two lines at Invermay New Zealand and then implanted one month later in Border Leicester x Merino ewes at UNE Armidale Australia. All sheep in this experiment were from these embryo transfer programmes.

3.2.2 Experimental design

A total of twenty-four mature sheep (12 rams and 12 ewes greater than 2.5 yrs of age at the start of the experiment) from fat and control selection lines were used in the experiment (Fennessy *et al.*, 1987). Ewes that were used in the experiment had successfully reared a lamb in the previous season, the lambs being weaned in February 1993. All sheep had been run at pasture ("Kirby" UNE Armidale) prior to the start of the experiment. The experiment was a 2x2x3 factorial design which allowed comparison of the effects of sex, genotype and feeding levels on changes in body composition with respect to body weight and time.

The sheep were shorn, vaccinated, drenched and moved into individual indoor pens (3x3 metres) two weeks prior to the start of the experiment. The sheep were weighed after a 24 hour fast and an estimate was made of the feed required to maintain liveweight using

an equation that predicted metabolisable energy needs as a function of metabolic weight, with scaling terms for both age and sex (SCA, 1990). Adjustments were made to the feeding level when an animal had a deviation of ± 2 kg in liveweight when weighed after a 24 hour fast on 4 occasions prior to the start of the experiment. Generally these adjustments were no more than ± 30 g/day. At the start of the experiment feed intakes to maintain liveweight had stabilised for all sheep. This level was defined as 100% of maintenance requirements for each individual.

All sheep were fed at a constant level, calculated initially at the maintenance level (100%), for 42 days (6 weeks). At the end of this period the sheep were scanned to estimate body composition after a period of liveweight maintenance. At the beginning of week 7, 16 animals were randomly selected on a stratified weight basis and fed at two levels below that required for live weight maintenance. The levels were 80% and 60% (2 animals from each sex/genotype subclass at each level) of the maintenance level. Feeding levels for these sheep remained at below maintenance for 16 weeks with animals scanned in week 9, 12, 15 and 21 of the experimental period. These 16 sheep were then fed at the original maintenance feeding level (100%) for a further 15 weeks to allow for a period of liveweight realimentation, during this time the sheep were scanned at 25, 30 and 36 weeks.

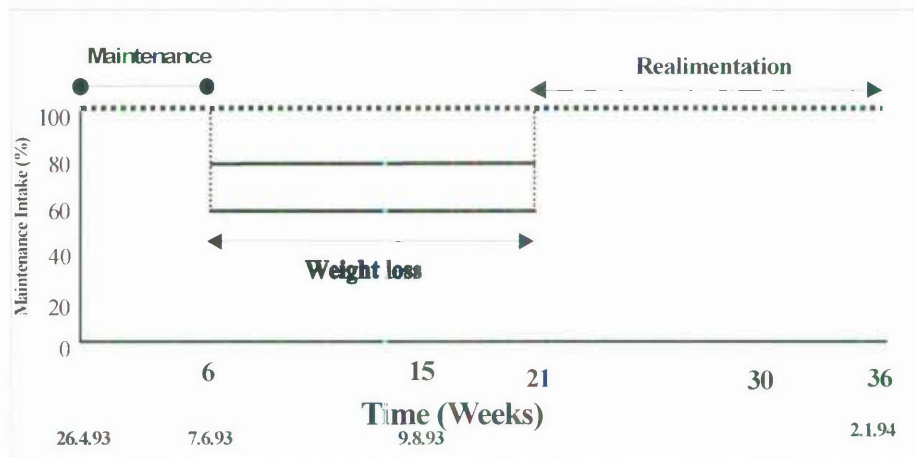


Figure 3.2.1 Feeding levels of the three treatment groups during the experimental period.

Four males and four females (two from each genotype) were fed at a constant feed level for the entire experimental period to provide a control and were scanned at the same

time intervals as those for the treatment groups. The experimental design is summarised in Table 3.2.1. and Figure 3.2.1.

Table 3.2.1 Timetable of events and scanning dates for the restricted and realimentation phase respectively.

| Week | Date | Event |
|------------------------------------|-------------|--|
| <u>Adjustment Phase</u> | | |
| Week -1 | 19.4.93 | All mature sheep scanned at 12/13 rib. Experimental sheep selected and placed in shed. Sheep drenched. |
| Week 0 | 26.4.93 | Control and Weight loss sheep placed at constant feed intake(maintenance). Sheep fed daily at 8.00am and weighed weekly prior to feeding for WL and for Controls. Sheep weighed and blood sampled weekly. |
| <u>Restriction Phase</u> | | |
| Week 6 | 7.6.93 | Sheep scanned (Scan 1). Sheep were randomly allocated to treatment Weight loss sheep placed on below maintenance feeding levels. Control animals at constant feed intake. |
| Week 9 | 28.6.93 | Sheep scanned (Scan 2). Management as above. |
| Week 12 | 19.7.93 | Sheep scanned (Scan 3). Management as above. |
| Week 15 | 9.8.93 | Sheep scanned (Scan 4). Management as above. |
| Week 21 | 20.9.93 | Sheep scanned (Scan 5). End of restriction phase. |
| <u>Realimentation phase</u> | | |
| Week 0 | 27.9.93 | All sheep fed daily at a constant level predetermined for weight maintenance at the start of the experiment. Sheep weighed and blood samples taken weekly. |
| Week 4 | 25.10.93 | Sheep scanned (scan 6). Management as above. |
| Week 9 | 29.11.93 | Sheep scanned (Scan 7). Management as above. |
| Week 13* | 22.12.93 | Control sheep scanned (Seasonality scan 9). Management as above. |
| Week 15 | 10.1.94 | Sheep scanned (Scan 8). End of Realimentation phase. |

3.2.3 Feeding and Handling Procedures

The sheep were fed once daily at 0800 hours for the entire experimental period with the following exceptions. On the day of weighing, the sheep were bled and weighed prior to feeding, with feeding commencing at 1000 hours. On scanning days, sheep to be scanned were removed from the shed after weighing for scanning and returned to the shed prior to 1600 hours at which time the feed for that day was given. Water was provided *ad libitum* from individual self waters in each pen during the experiment. At scanning times hooves were trimmed to avoid feet problems.

Sheep were weighed weekly using the same electronic scale on all occasions. A standard weight was weighed periodically to check the repeatability of the scale. The sheep were shorn at 3 month intervals commencing 2 weeks prior to the start of the

experiment. Wool growth per week was determined from a linear function based on the weight of fleece at each shearing time. Liveweights were adjusted to a fleece free full body weight for all analyses.

A 10ml blood sample was taken at weighing from each sheep into lithium heparin (Vacutainer® Bectin Dickinson) via jugular vena puncture using 20gx1½ needles (Vacutainer® Bectin Dickerson). Blood samples were stored immediately in ice and then centrifuged at 3000 rpm, the plasma was removed and stored in two duplicate 5ml vials at -20°C.

3.2.4 Diet

The sheep were fed a pelleted ration (Fielders Agricultural Products, Tamworth, Australia; Table 3.2.2). The feed was weighed into individual buckets in amounts for 4 and 3 days respectively.

Table 3.2.2 Components and dry matter composition of the experimental pellet.

| Component | (g/kg) | Dry Matter Component | (g/kg) |
|--------------|--------|----------------------|-------------|
| Wheat | 300 | Organic Matter | 93.2 |
| Soybean meal | 107 | Crude protein | 16.9 |
| Lucerne meal | 573 | Metabolisable energy | 10.2(MJ/kg) |

* To 1000 kg of this ration was added: 10kg finely ground limestone, 2.5kg NaCl and 8kg of a vitamin mineral premix (8kg of the mineral premix contains 5.916kg sodium sulphate, 0.5kg Ammonium sulphate, 0.5kg Ammonium chloride, 12x10⁶ i.u. Vitamin A, 5x10⁶ i.u. Vitamin D3, 10g Vitamin E, 1g Cobalt, 1g Iodine, 25g Molybdenum, 0.1g Selenium, 15g Iron, 20g Manganese and 50g Zinc

3.2.5 Animal Health.

On 28/8/93 (week 11) ram 117 died as a result of siliceous urolithius. Several other rams were identified as having similar symptoms (Stewart *et al.*, 1991). After consultation with the Armidale Regional Veterinary Office NSW Department of Agriculture at this point in the experiment, ammonium chloride (NH₄Cl) was added to the feed for each sheep at 4% of the weight of feed offered per day until the experiment was completed. An additional ram (21 Control line) was introduced to the experiment at this time. The ram was weighed and an estimate made of feed intake required to maintain liveweight in line with the procedure of the other animals in the experiment. This ram was

fed a constant feed level for the duration of the experiment and was scanned at scan times 5, 6, 7 and 8.

Ram 19 (Fat line) was diagnosed as having a minor spinal disorder, this disorder lead to periods of chronic arthritis during the experiment. On several occasions Norocillan L.A.® was given to this sheep intramuscularly. This ram died on the 19/11/93. Ram 107 (Control line) died on the 4/1/94 of rumen peritonitis after ingesting a short length of wire.

On the 31/1/94 several sheep developed severe rumen acidosis and bloat and by the following day all sheep refused feed. Under advice from the University ACEC veterinary officer all sheep were drenched with 500mls of water containing 100g NaHCO₃ at 1100, 1500 and 1900 hours. By the 2/2/94, six ewes had died and the experiment was terminated with sheep being transferred to pasture. To date, the cause of these deaths has not been identified, although kidney failure occurred in most of the surviving animals.

In order to test for possible toxins in the feed an experiment was conducted for a period of 21 days involving 18 mice that were fed *ad libitum* the same pelleted ration as the sheep (PACEC 940027). At the end of this experiment all mice were healthy and a subset of mice that were slaughtered failed to show any abnormal effects in either the liver or the kidney. The conclusion was that there were no toxins in the feed at that time. A further experiment involving four rumen fistulated sheep was conducted, where the sheep were fed the pelleted ration daily at maintenance levels for four weeks. Water consumption and rumen pH and NH₃ levels remained within normal ranges for these animals. To date, no satisfactory conclusion has been reached as to why the sheep died.

3.2.6 CAT-Scanning procedure

On scanning days, the liveweights of animals were recorded prior to scanning. All animals were scanned using a whole body X-ray computer aided tomography system (Hitachi CTW-430 Computed Tomography system). The group were scanned over a three day period in the weeks of scanning (see Table 3.2.1), with each animal scanned on the same day (Tuesday, Wednesday or Thursday) Sheep were transported to the Cat-scanner-

building of the University of New England and prior to scanning the animals were restrained on a fibreglass bed and sedated with Acepril® (0.1 ml/ 10kg liveweight; Troy laboratories, Australia) when necessary. On average, total scan time for each animal was approximately 35 minutes.

For each sheep tomographs were made of the whole body from a randomly positioned point set behind the rump (distal to the proximal hind limb muscles), at distances of 40 mm until the end point of the scanning at the 4th/5th cervical vertebrae. On average 25 scans were taken from each animal.

For the purposes of estimation of body composition *in vivo* each tomograph was divided into eleven tissue depots using the program CATMAN (Thompson and Kinghorn, 1992). Bone included all tissues with the equivalent grey scale (201-254) to that of bone. Tissues with the equivalent density to fat (40-115) were separated into five depot groups being:

- * Subcutaneous fat - tissue highlighted as fat that lay immediately beneath the skin and above layers of muscle tissue, excluding fat associated with the udder or scrotum.

- * Intermuscular fat - tissue highlighted as fat which lay between two layers of skeletal muscle.

The combined weights of these two tissues can be defined as carcass fat.

- * Internal fat - tissue highlighted as fat which lay within the abdominal or thoracic cavity and included the omentum, mesentery, kidney and channel, and heart fat depots.

- * Udder/Scrotal fat - tissue highlighted as fat that surrounded or was associated in the structure of the scrotum for rams and the udder for ewes.

- * A separate independent measure of all total fat tissue was made and defined as total fat.

Tissues with equivalent density to lean (116-200) were separated into two depots being

* Carcass muscle - tissue highlighted as lean which would be considered to form part of the carcass. Muscle tissue of the tail and skin (note that wool was not highlighted and therefore was not included in any group for analysis) was included in this depot.

* Visceral lean - tissue highlighted as lean and separate from that defined as the carcass. Included alimentary tract, abdominal and thoracic organs, and udder or scrotal lean tissue.

Contents of the rumen, reticulum, omasum, abomasum, caecum, colon and bladder were excluded from the images. Soft tissue within the bone was measured and contained both fat and lean tissue within the medulla of the bones. A separate estimate of the fleece free empty body weight (empty body weight) was made and included all body tissues of fat, lean and bone excluding the contents of the rumen, reticulum, omasum, abomasum, caecum, colon and bladder.

Image processing, changed with the availability of AUTOCAT (Jopson *et al.*, 1995). All images from scan 4 until the end of the experiment (Scan 8) were analysed using AUTOCAT. Briefly, the two dimensional tomographs were converted into bitmap format and were edited through a commercial image package (Picture Publisher 5.0[®]; Micrografx), so that two additional images were created. The first image (A) included all body tissues excluding the contents of the rumen, reticulum, omasum, abomasum, caecum, colon and bladder. From this image areas for total fat, total lean and bone were calculated. Areas for carcass fat (sum of subcutaneous and intermuscular fat depots), and carcass muscle were obtained from a second modified image (B) that contained all tissues from the carcass, by excluding tissues of the internal organs and internal fat depots. By difference the areas for internal fat and visceral lean were obtained. The results from this procedure were tested against the original results for CATMAN on all animals in scan 4 with an R^2 of 0.94. Once areas had been calculated the weight of each tissue was determined using the same procedure as that for CATMAN (Thompson and Kinghorn, 1992).

Briefly, the total weight of each component was estimated from the multiplication of the volume of the tissue and the density of the tissue. The volume of the tissue was determined using *Cavalieri's principal* by multiplying the sum of the areas of each tissue by the respective distance between each scan (40mm in this experiment). Roberts *et al.* (1993) indicated that the use of the *Cavalieri* principal together with modern non-invasive scanning techniques of either magnetic resonance imaging (MRI) or CAT-scanning easily and efficiently estimated body components *in vivo*. Densities of respective tissues were obtained by using the regression equation of Fullerton (1980) which relates Hounsfield units (μ) to tissue density by the following function.

$$\text{density (g/cm}^3\text{)} = 1.0062 + (\text{mean tissue Hounsfield unit } (\mu) \times 0.000106)$$

3.2.7 Statistical methods

The ability to obtain sequential measurements of body composition in all sheep through use of the cat-scanning procedure, provided the opportunity to fit repeated measures analysis to the individual body components against time. This allowed the effects of levels of maintenance (100%, 80%, 60%; treatment), sex and line on the priorities for mobilisation and realimentation of tissues in mature sheep over the restricted and realimentation phase to be identified on a within animal basis. In order to quantify changes in the individual components over time, the weights of the individual components measured on days 21, 42, 63 and 105 of the restriction period and on days 28, 63, 105 of the realimentation phase were analysed as repeated measures across time.

The data in this experiment were the seven individual body components for the 25 individual animals subject to the fixed effects of three treatments, two sexes and two lines. Various components or responses were measured on each animal for a maximum of 8 possible sampling times. Random effects models are frequently used in longitudinal settings because these effects are dominant in the data error structure (Diggle *et al.*, 1994). Variograms (Diggle, 1990) were constructed for the residual correlations against time for all body components that were analysed. These variograms indicated that the random effects due to the between animal variation were dominant in the error structure and that the effect of serial correlation between the repeated measurements were minimal. Random

effects models assumes that the correlations between repeated measures for an individual animal arise through the sharing of a underlying growth curve composed of unknown variables (Diggle *et al.*, 1994)

The analysis of the present experiment used the techniques of Cullis *et al.* (1996) and the accompanying S-plus functions to incorporate cubic smoothing splines into a linear mixed model. The use of the spline techniques was considered preferable to a non-linear method because the experimental design with an abrupt change in feeding levels did not easily fit into a parametric setting.

3.2.7.1 Modeling procedure

The first step in the analysis of each component (y) was to fit a saturated model as follows;

$$Y_{ijkl} = \mu + T_i + S_j + L_k + TS_{ij} + TL_{ik} + SL_{jk} + TSL_{ijk} + D_l + F(D_l) + R(D_l) + TD_{il} + T_iF(D_l) + T_iR(D_l) + DS_{jl} + S_jF(D_l) + S_jR(D_l) + D_lS_{ijl} + TS_{ij}F(D_l) + TS_{ij}R(D_l) + Animal_{ijk}$$

where

Y_{ijkl} is the response for treatment i , sex j , line k and day l .

μ is the overall mean

T_i is the i^{th} treatment effect

S_j is the j^{th} sex effect

L_k is the k^{th} line effect

D_l is the linear time term that was scaled to centred mean by (time-mean(time))/ $\sqrt{\text{variance of time}}$

$F(D_l)$ is the non-linear component of the model that is assumed to have the form of a cubic spline

$R(D_l)$ is the random time effect that reflects the lack of fit from the corresponding fitted cubic smoothing spline

$Animal_{ijk}$ is the random animal effect

The variance components (random effects; interactions with either spline time or random time) were then tested for significance by successively dropping their terms from the model and assessing the change in the log-likelihood statistic (i.e. $\Delta 2l \sim \chi^2_{(1)}$). This procedure tests the hypotheses as to whether the inclusion of a particular variance component significantly improves the model fit. When the appropriate variance component model has been selected the significance of the fixed effects ($P < 0.05$) was

determined using a two-tailed t-test. The significant effects as well as their non-significant lower order terms were then included in the final model. Treatment was fitted as Helmert contrasts (Chambers and Hastie, 1992). Time was fitted as standardised time.

As the "shape" of the response for each component over the experiment period, rather than the population mean is of interest, graphs of the significant fixed effects relating to slope and the random spline slopes (\hat{y}) and their 95% confidence limits are presented. The components of the fixed effects and the random terms relevant to the slope ("shape") are shown as a deviation from zero (the overall mean) with respective 95% confidence intervals for each spline calculated as follows

$$\hat{y} = x\hat{\beta} + Z\hat{u}$$

$$\text{var}(\hat{y}) = x \text{var}(\hat{\beta}) x^T + Z \text{var}(\hat{u}) Z^T + 2 * \text{cov}(x\hat{\beta} + Z\hat{u})$$

where

x is the design matrix of the fixed effects

Z is the design matrix of the random effects

$\hat{\beta}$ is the estimated fixed effects

\hat{u} is the estimated random effects

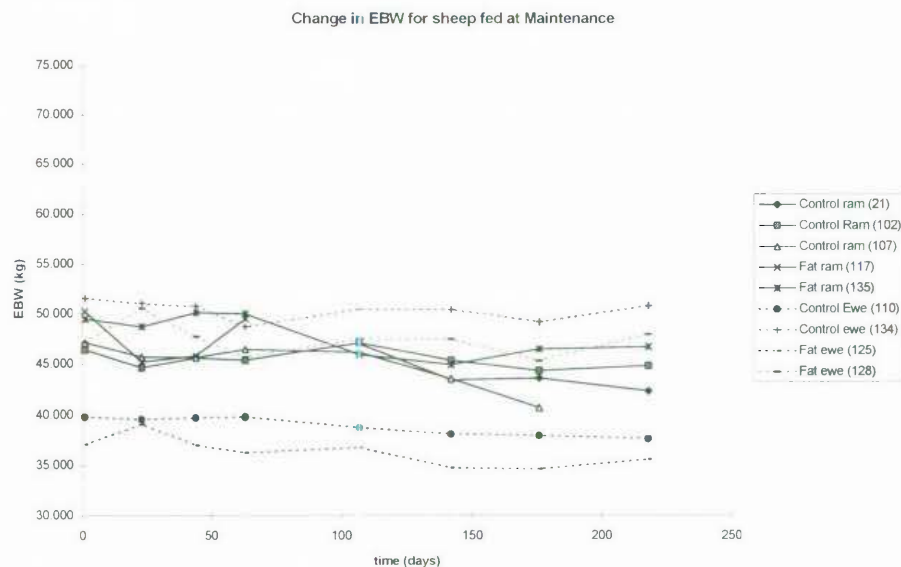
3.3 Results

The mean empty body weights (EBW) and component weights for carcass muscle, visceral lean and total fat (\pm standard error of the mean (sem)) for the sex and line subclasses are presented in Table 3.3.1. There were no initial treatment differences at the start of the experiment.

Table 3.3.1 Mean fleece free empty body weight (EBW), carcass lean, viscera and total fat weights (\pm sem) at the start of the experimental period for control and fat rams and ewes.

| | Line | | | |
|-------------------------------|---------|-------|-------|-------|
| | Control | | Fat | |
| | Rams | Ewes | Rams | Ewes |
| Number | 6 | 6 | 6 | 6 |
| Total Fat (kg) | 9.30 | 16.34 | 15.29 | 16.71 |
| sem | 1.00 | 1.47 | 2.84 | 1.11 |
| Carcass muscle (kg) | 25.15 | 19.27 | 24.74 | 17.10 |
| sem | 1.82 | 0.62 | 1.22 | 0.55 |
| Visceral lean (kg) | 7.84 | 5.74 | 6.98 | 4.64 |
| sem | 0.31 | 0.27 | 0.33 | 0.16 |
| Empty body weight (kg) | 47.94 | 45.64 | 52.24 | 42.00 |
| sem | 3.17 | 1.95 | 3.83 | 1.40 |

The actual empty body weights of each individual animal within the three treatments over the entire experimental period are shown in Figure 3.3 for sheep fed at 100%, 80% and 60% of maintenance respectively. As can be seen graphically, the general pattern of response for each treatment x sex group was masked by the dominance of the individual animal response.



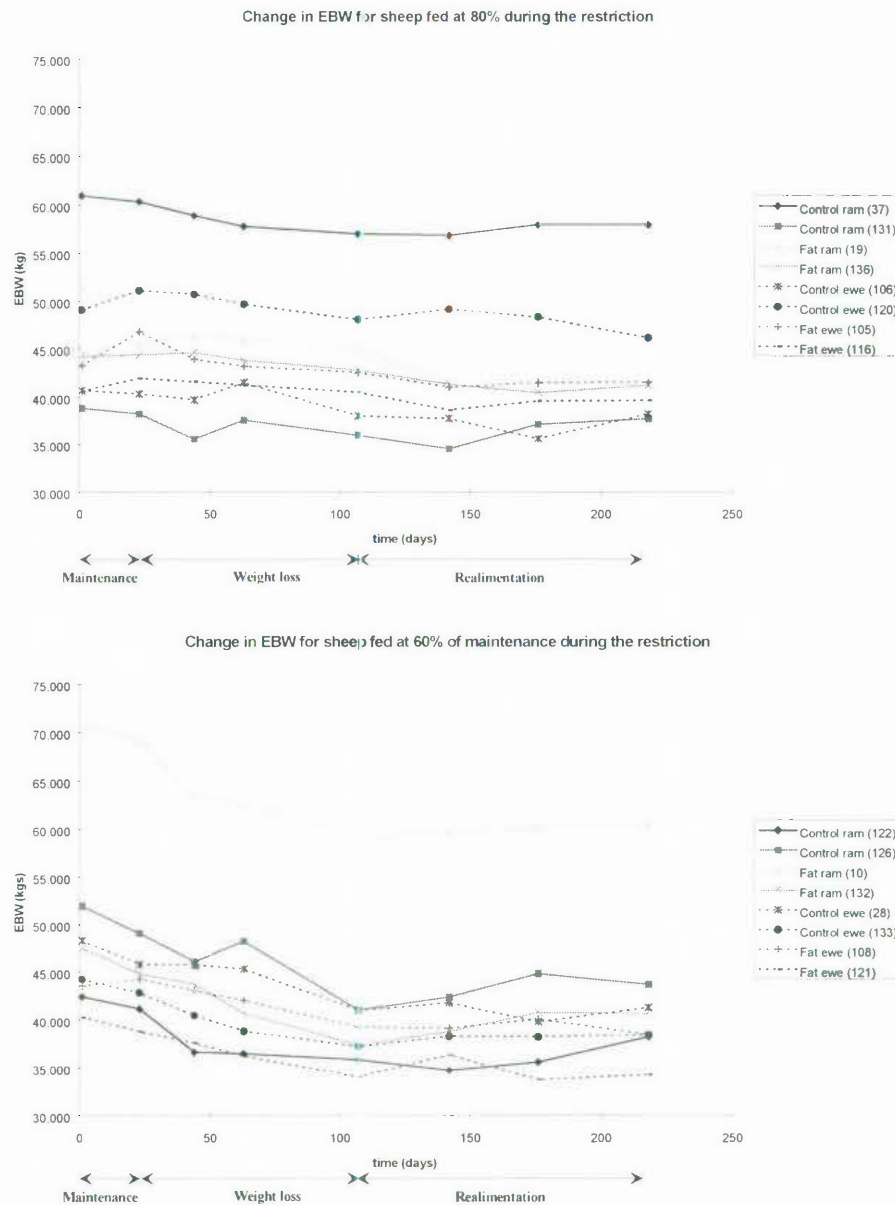


Figure 3.3 Changes in empty body weight (EBW; kg) for all individual sheep over the restriction and realimentation phases of the experiment.

The coefficients of the significant fixed effects, together with their t-value and significance of the t value for the final models for each of seven body components are shown in tables 3.3.2., 3.3.4 and 3.3.5. The significant random effects ($P < 0.05$) for the final models of each of the seven body components are shown in Table 3.3.3. There were no line*linear time, line*spline time or line*random time effect in the final models for all body components analysed. This indicated that there was no significant effect of total body fatness (as assessed by the line comparison) on the patterns of tissue depletion or deposition during the weight loss or realimentation periods.

Table 3.3.2 Significance and t-values for the overall mean (μ (kg)), deviations for fixed effects from the overall mean and deviations for the linear time effects for empty body weight.

| | | Empty Body Weight | | |
|-----------|---------------------|--------------------------|---------------------|----------------|
| | Overall Mean | Fixed effects | Time effects | t-value |
| | (kg) | | | |
| (μ) | 44.307 | | | 31.192*** |
| | | Treat1 | | 0.171 |
| | | Treat2 | | 0.105 |
| | | Sex | | 1.493* |
| | | Treat1*sex | | -0.402 |
| | | Treat2*sex | | -0.251 |
| | | | Time | -12.784*** |
| | | | Treat1*time | 3.712*** |
| | | | Treat2*time | 2.861*** |
| | | | Sex * time | -1.008 |
| | | | Treat1*sex*time | -1.412* |
| | | | Treat2*sex*time | 1.867** |

* P <0.1

** P<0.05

*** P<0.01

Treatment effects of 60, 80 and 100% were fitted as Helmert contrasts of treat1 and treat2.

Table 3.3.3. Significant variance components (Random effects) of the final models for the seven body components.

| Component | EBW | Carcass muscle | Visceral lean | Total Fat | Carcass Fat | Internal Fat | Bone |
|--|------------|-----------------------|----------------------|------------------|--------------------|---------------------|-------------|
| Random effects | | | | | | | |
| Spline time F(D _i) | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| Random time R(D _i) | | ✓ | | ✓ | | | |
| Treatment:Spline time T _i F(D _i) | ✓ | ✓ | ✓ | ✓ | ✓ | | |
| Treatment:random Time T _i R(D _i) | | ✓ | | | | | |
| Sex :spline time S _j F(D _i) | ✓ | ✓ | | ✓ | | | |
| Sex: random time S _j R(D _i) | ✓ | ✓ | | | | | |
| Treatment:sex :spline time TS _{ij} F(D _i) | ✓ | ✓ | | | | | |
| Treatment:sex: random time TS _{ij} R(D _i) | | ✓ | | | | | |
| animal | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

✓ Indicates significant variance components (random effects) : t P<0.05

3.3.1 Empty body weight

The deviations of the spline and random time components over time for EBW with the 95% confidence intervals for the treatment * sex subgroups are shown in Figure 3.3.1. The significant effects of treatment * sex * spline time and treatment * sex * random time (Table 3.3.3) indicated that the patterns of response for the sexes deviated within treatment groups. Rams fed at maintenance lost approximately 3 kg over the 218 days of constant feeding. Ewes fed at maintenance also had slight decline in EBW, although the weight at the end of the restriction did not differ significantly to that at the start.

There was a marked sex effect on EBW for those animals fed at 80% of maintenance. The rams showed a curvilinear decline in EBW of approximately 2.5 kg over the course of the restriction phase or 5.6% of the initial weight (44.3 kg; Table 3.3.2) and a slight increase during the period of realimentation. The ewes fed at 80% of maintenance during the restriction phase maintained weight initially and then mobilised body tissue during the later stages of the restriction and into the realimentation phase. During the final period of the realimentation EBW remained stable for this group. For both the sexes, the final EBW at the end of the realimentation was significantly less than the initial starting weight.

For sheep fed at 60% of maintenance the pattern of change in EBW was similar for rams and ewes, in that both lost EBW over the course of the restriction. The rate of loss was greater in rams than in ewes which is reflected in the respective losses of approximately 8.8 and 5.3 kg or 20 and 12% respectively of the initial weight. During the realimentation phase rams had a slight increase in EBW, whilst the ewes showed no change in EBW. For both groups the final weight reached at the end of the realimentation period was significantly less than the starting weight.

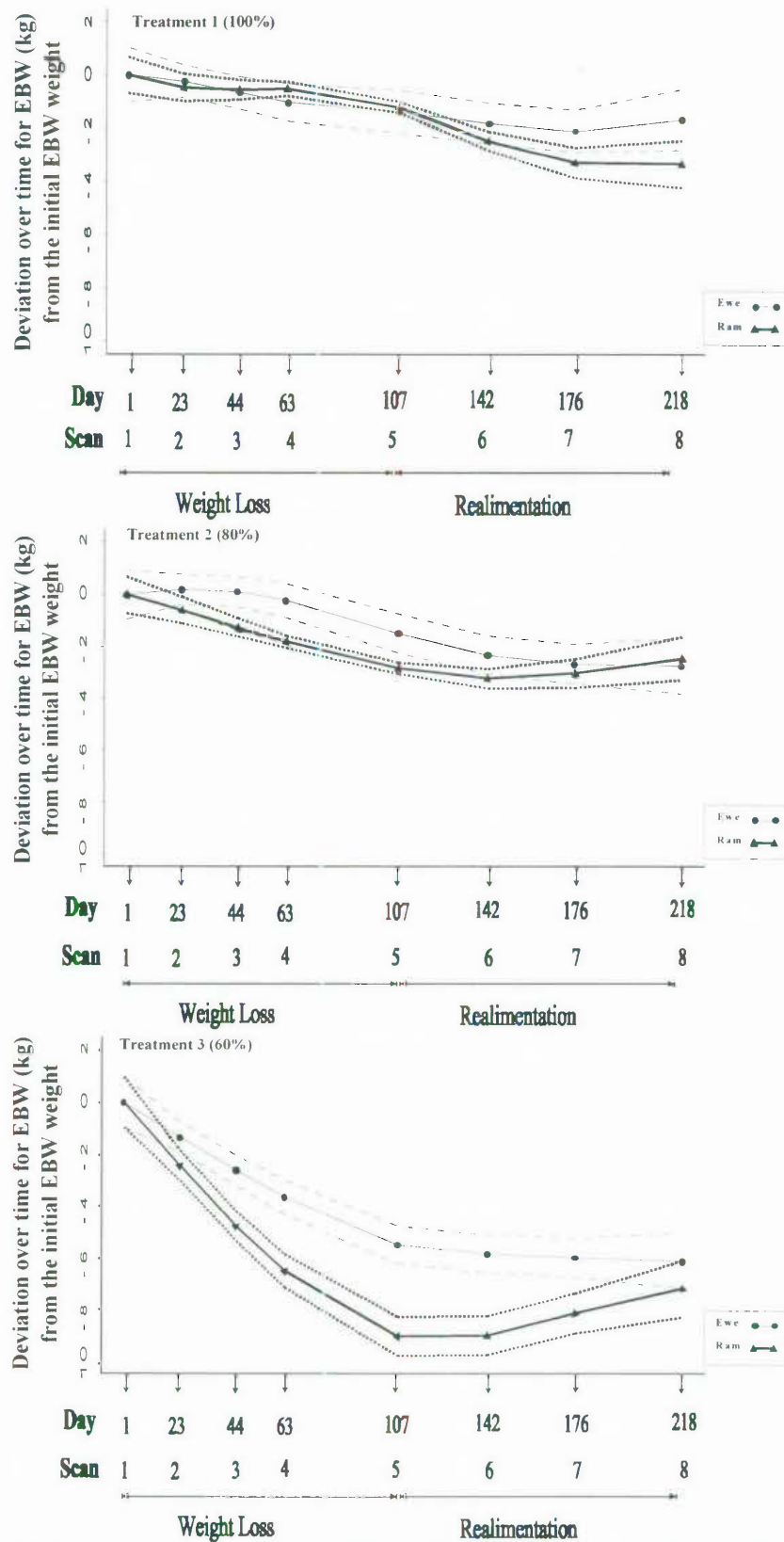


Figure 3.3.1 Deviations for empty body weight (kg) from initial empty body weight and the 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

3.3.2 Carcass muscle weight

Rams fed at maintenance lost carcass muscle during the experimental period. By the end of the experiment there was no significant difference in the weights of carcass muscle in either of the three treatment groups for the rams. This indicates that a factor other than feeding level may have been responsible for the decline in carcass muscle weight that was observed for mature rams. There was no change in carcass muscle mass for the ewes fed at maintenance over the entire experimental period. As with the rams, the final weights of carcass muscle did not differ significantly for all treatment groups for the ewes.

There was a significant sex effect on the patterns of carcass muscle mobilisation for sheep fed at 80% of maintenance during the restriction period. Rams lost carcass muscle at a slow rate over the restriction period, whilst ewes did not mobilise significant amounts of carcass muscle. The loss for the rams represented 6.4% of the initial overall mean of 20.4 kg (Table 3.3.4). During realimentation there was no evidence of regain of lost carcass muscle for either sex and at the end of the realimentation period there was no significant difference in the weight of carcass muscle from that at the start of the experiment for the ewes. In contrast the rams had lower weights of carcass muscle at the end of the experiment than at the start.

There were significant random effects of treatment * sex * spline time for carcass muscle over the experimental period (Table 3.3.3). Both rams and ewes fed at 60% of maintenance lost carcass muscle over the early phase of the restriction period as shown in Figure 3.3.2. The rate of change during the final phase of the restriction (day 63-105) for both groups approached zero. The respective losses for the rams and ewes of the initial weight were 12.3% and 8.6%. During the realimentation period there was little gain in carcass muscle for either sex and at the end of the realimentation period both groups still had appreciably lower weights of carcass muscle than at the start of the experiment.

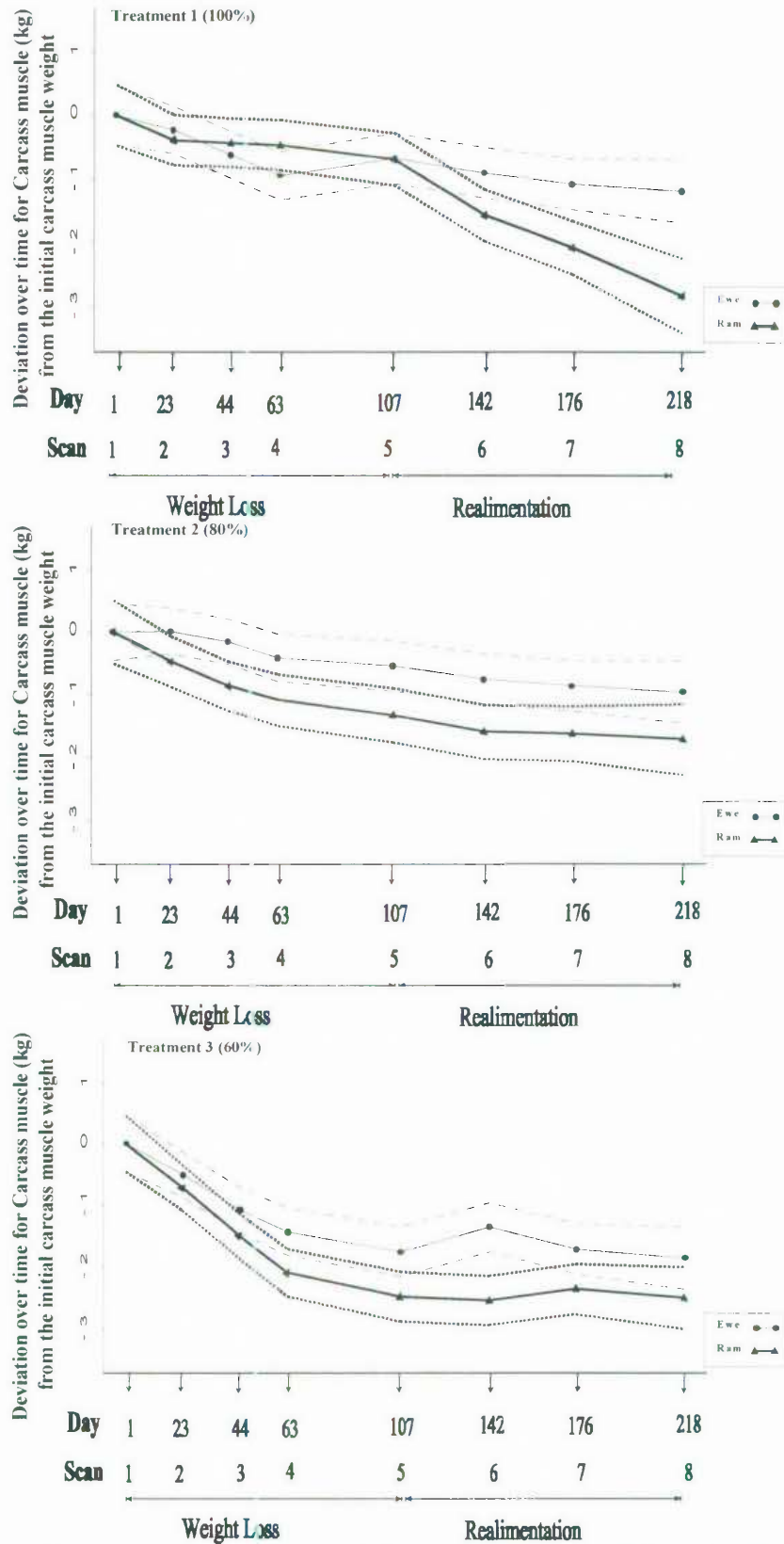


Figure 3.3.2 Deviations for carcass muscle (kg) from initial carcass muscle weight and 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

3.3.3 Visceral lean weight

The final model of the significant random effects for visceral lean indicated that there was a treatment * spline time effect (Table 3.3.3). Visceral lean weights did not differ significantly over the experimental period for sheep fed at either maintenance or 80% of maintenance during the restriction period. As there was no time effects for these treatment groups only the 95% confidence intervals are shown for the deviations over time of visceral lean for sheep fed 60% of maintenance (Figure 3.3.3). For sheep fed at 60% of maintenance, 10% of the initial visceral lean weight was mobilised over the restriction period. However, in contrast to results shown for other body tissues, there was a complete compensation of visceral tissue that had been previously mobilised during the realimentation period.

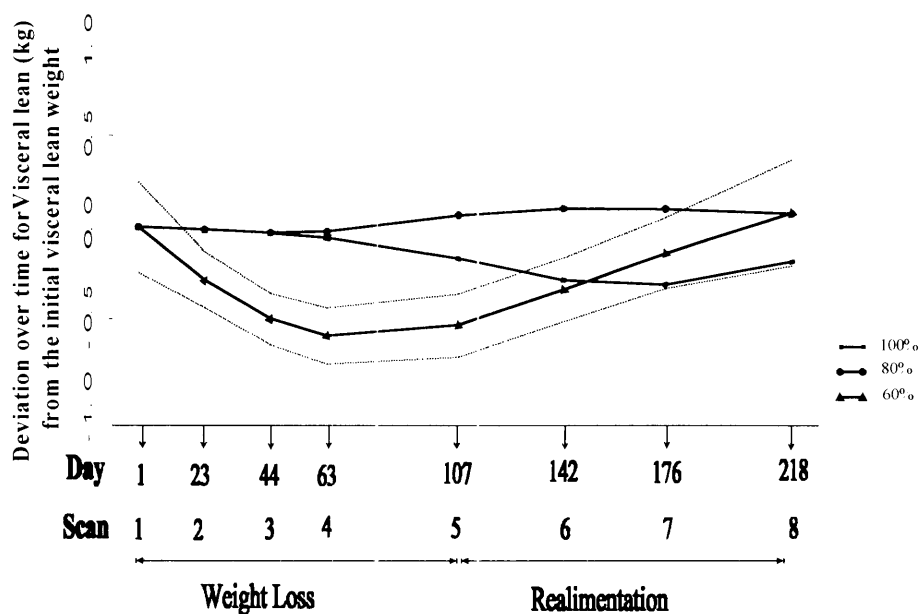


Figure 3.3.3 Deviations for visceral lean (kg) from initial visceral lean weight and 95% confidence limits (dotted lines for the 60% treatment group) for the three treatment groups during the restriction and realimentation phases of the experiment.

3.3.4 Total fat weight

There was no obvious pattern of change in the total fat weight of either rams or ewes fed at maintenance over the experimental period. For both groups of sheep fed at below maintenance during the restriction period there was a direct effect of sex on the

patterns of mobilisation and deposition as indicated by the significance of the treatment *sex * spline time random effect in the final model for total fat (Table 3.3.3).

Rams and ewes fed at 80% of maintenance preferentially conserve total body fat during the initial phase of the restriction period, but both sexes mobilised total fat over the final stage of the restriction period (day 63-107). At the end of the restriction phase rams had lost 11% of their initial fat weight and ewes had lost 8% of their initial fat weight (Table 3.3.4). During the realimentation period, both sexes regained total fat, although the gain for the rams was greater. At the end of the experiment both sexes had demonstrated the ability to compensate for total fat lost during the restriction period.

In contrast, the rams and ewes fed at 60% of maintenance mobilised significantly greater weights of total fat over the restriction period than the sheep fed at maintenance (Figure 3.3.4). Rams showed a greater overall loss of total fat than ewes during the restriction with respective losses of 34% and 26% of the initial overall mean (13.7 kg; Table 3.3.4). As indicated above, there was a greater level of compensation of total fat for rams during the realimentation period than for ewes and by the end of the realimentation there was no difference in the weights of total fat for either sex. In contrast to the results for sheep fed at 80% of maintenance, it appears that mature sheep restricted at 60% of maintenance did not have the ability to recover lost total fat tissue when re-fed at maintenance levels during a period of realimentation.

Table 3.3.4 Significance and t-values for the overall mean (μ (kg)), deviations for fixed effects from the overall mean and deviations for the linear time effects for carcass muscle, visceral lean and total fat.

| <u>Body Component</u> | <u>Mean (kg)</u> | <u>Fixed effects</u> | <u>Time effects</u> | <u>t-value</u> |
|------------------------------|------------------|----------------------|---------------------|----------------|
| <u>Carcass muscle</u> | | | | |
| Mean (μ) | 20.351 | | | 37.455*** |
| | | Treat1 | | -0.469 |
| | | Treat2 | | 0.994 |
| | | Sex | | 5.430*** |
| | | Treat1*sex | | -0.277 |
| | | Treat2*sex | | 0.865 |
| | | | Time | -11.0*** |
| | | | Treat1*time | -0.769 |
| | | | Treat2*time | 1.697** |
| | | | Sex*time | -3.128*** |
| | | | Treat1*sex*time | -1.620* |
| | | | Treat2*sex*time | 0.980 |
| <u>Visceral lean</u> | | | | |
| Mean (μ) | 5.998 | | | 43.346*** |
| | | Treat1 | | -0.343 |
| | | Treat2 | | 0.228 |
| | | Sex | | 6.945*** |
| | | Line | | 3.580*** |
| | | | Time | -1.347* |
| | | | Treat1*time | -2.163** |
| | | | Treat2*time | 0.748 |
| | | | Sex*time | 1.558* |
| <u>Total Fat</u> | | | | |
| Mean (μ) | 13.705 | | | 14.905*** |
| | | Treat1 | | 0.800 |
| | | Treat2 | | -0.585 |
| | | Sex | | -2.613*** |
| | | Line | | -1.923** |
| | | Treat1*sex | | -0.173 |
| | | Treat2*sex | | -1.084 |
| | | | Time | -4.668*** |
| | | | Treat1*time | 9.170*** |
| | | | Treat2*time | 2.530*** |
| | | | Sex*time | 0.817 |
| | | | Treat1*sex*time | -1.102 |
| | | | Treat2*sex*time | 1.938** |

* P < 0.1

** P < 0.05

*** P < 0.01

Treatment effects of 60, 80 and 100% were fitted as Helmert contrasts of treat1 and treat2.

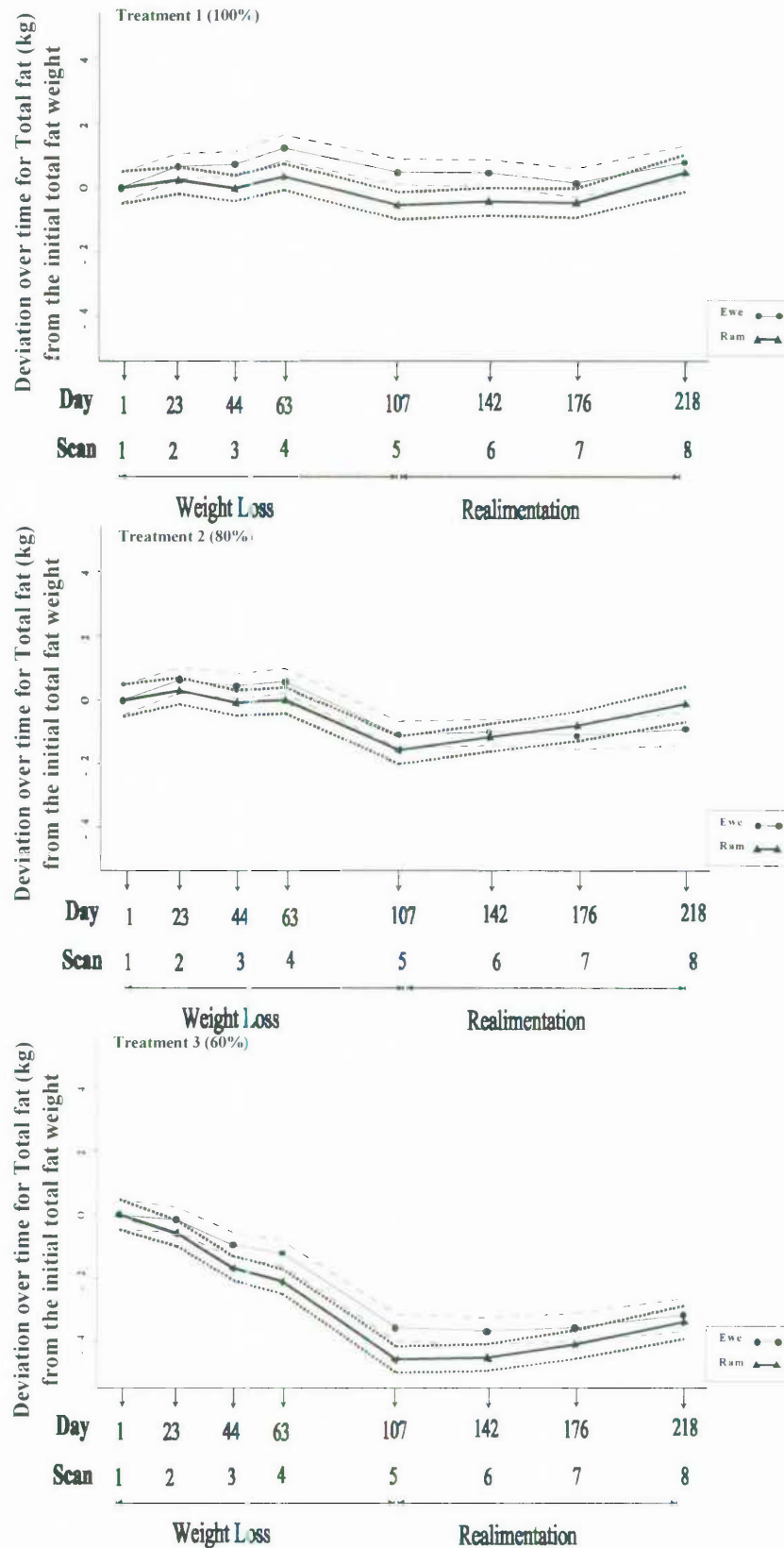


Figure 3.3.4 Deviations over time for total fat (kg) with 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

3.3.5 Carcass fat weight

There was a significant treatment * spline time effect for carcass fat (Table 3.3.3) over the experimental period (refer Figure 3.3.5.), this indicated that the response for sexes did not deviate within treatment groups. The patterns of change in carcass fat for the treatment sub-groups mirrored those for total fat. Sheep fed at maintenance deposited carcass fat over the experimental period, although as shown in Figure 3.3.5 the periods of deposition occur at the start and near the end of the experimental period. Sheep fed at 80% of maintenance conserved carcass fat in the early stages of the restriction period and as shown for total fat, carcass fat was mobilised over the final stages of the restriction. In contrast to the results obtained for total fat, sheep deposited carcass fat during the realimentation period resulting in complete compensation of lost tissue.

Carcass fat was mobilised at a constant rate over the restriction period for sheep fed at 60% of maintenance, with a loss of approximately 25% of the initial carcass fat weight. During the realimentation period, where the sheep were re-fed to original maintenance levels, only 50% compensation of lost carcass fat tissue occurred (Figure 3.3.5).

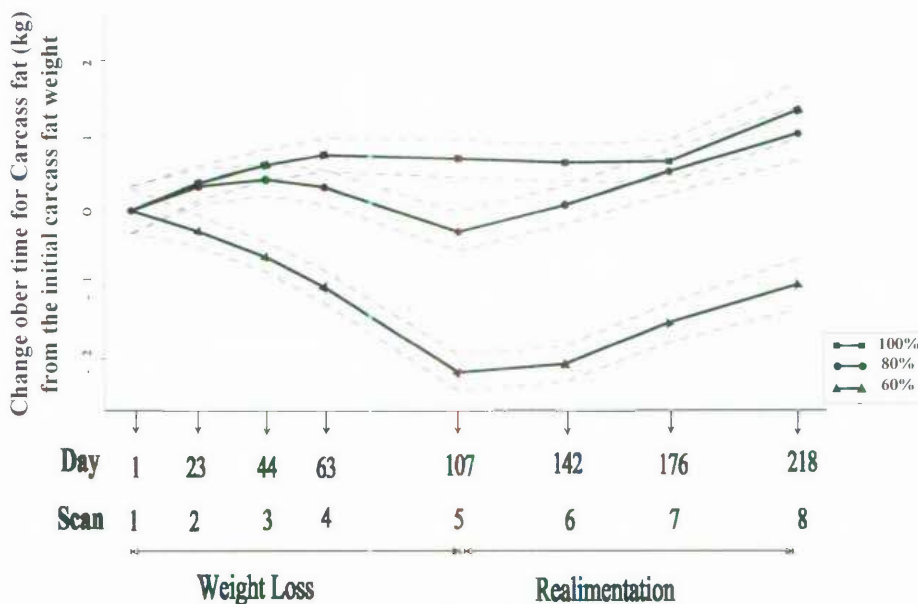


Figure 3.3.5 Deviations for carcass fat (kg) from initial carcass fat weight with 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

Table 3.3.5 Significance and t-values for the overall mean (μ (kg)), deviations for fixed effects (kg) from the overall mean and deviations for the linear time effects for carcass fat and internal fat depots.

| Fat Depot | Mean (kg) | Fixed Effects | Time effects | t-value |
|---------------------|-----------|---------------|-----------------|------------|
| Carcass Fat | | | | |
| Mean (μ) | 9.416 | | | 12.847*** |
| | | Treat1 | | 0.853 |
| | | Treat2 | | -0.458 |
| | | Sex | | -1.966** |
| | | Line | | -1.814** |
| | | Treat1*sex | | -0.150 |
| | | Treat2*sex | | -0.994 |
| | | | time | -5.350*** |
| | | | Treat1*time | 6.934*** |
| | | | Treat2*time | 2.249** |
| | | | Sex*time | 0.010 |
| | | | Treat1*sex*time | -1.271 |
| | | | Treat2*sex*time | 1.507* |
| Internal Fat | | | | |
| Mean (μ) | 4.194 | | | 21.648*** |
| | | Treat1 | | 0.753 |
| | | Treat2 | | -0.962 |
| | | Sex | | -4.671*** |
| | | Line | | -2.113** |
| | | | time | -13.317*** |
| | | | Treat1*time | 4.156*** |
| | | | Treat2*time | 1.752** |

* P < 0.1

** P < 0.05

*** P < 0.01

Treatment effects of 60, 80 and 100% were fitted as Helmert contrasts of treat1 and treat2.

3.3.6 Internal fat weight

In contrast to the results obtained for other tissues of the body, there were no random effects on the internal fat component over the experimental period. The nature of the significant linear time* treatment effect for internal fat is shown in Figure 3.3.6. There was a decline in the weight of internal fat for all three treatment groups over the entire experimental period. The rate of decline was greater as the level of the restriction increased. For the sheep fed at 60% of maintenance 38% of the initial internal fat weight was lost over the entire experimental period. Whilst only 18% for the sheep fed at 80% of the restriction and 12% for of the initial internal fat weight was lost for sheep fed at maintenance.

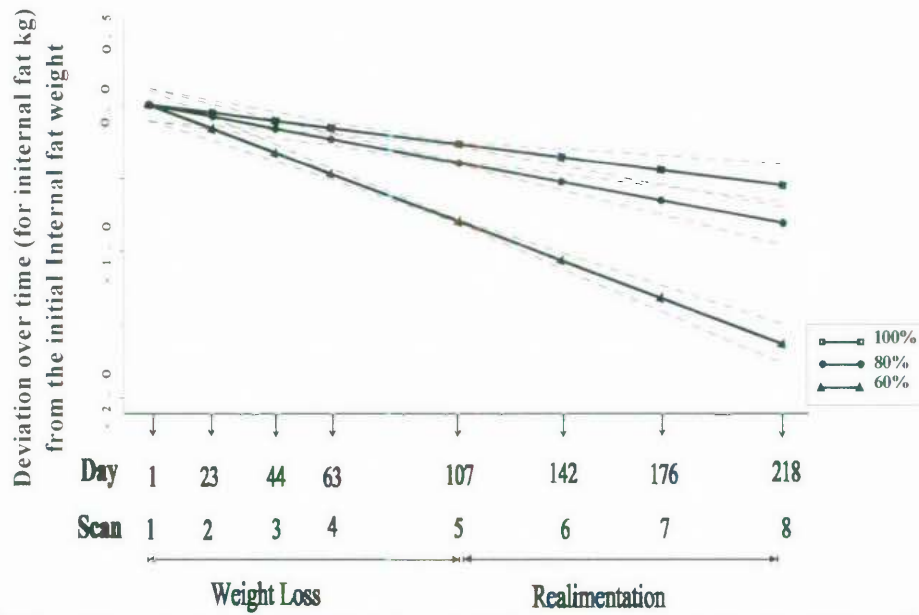


Figure 3.3.6 Deviations for internal fat (kg) from initial internal fat weight with 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

4.3.7 Bone

As shown in Table 3.3.3, there were no significant effects of either treatment, sex or line with time on the weight of bone over the experimental period. There were no significant time effects on bone over the experimental period. This indicated that the differences in the actual weights of bone at the start of the experiment remained consistent for the entire experimental period.

3.4. Discussion

3.4.1 Sheep fed at maintenance

Previous weight loss studies in ruminants have relied on describing the mobilisation of tissues from serial slaughter designs, or from the use of indirect methods based on estimating total body water from isotope marker dilution techniques and relating this estimate to total body fat. Changes in specific tissues have either been quoted as a function of time, as a percentage of the initial value, or more commonly as a function of body weight by the allometric equation ($Y=AX^b$). In this experiment the ability to accurately measure the changes in body composition over time for individual animals provided an unique approach to characterising the contribution of catabolism of specific tissues to meet an energy deficit induced by feeding at below maintenance levels.

The comparison of a randomly bred line with the line selected for an increase in backfat thickness (Fennessy *et al.*, 1987) provided the opportunity to evaluate the hypothesis that the weight loss patterns observed in either growing or mature animals, are primarily dependent on the energy stores prior to the period of feed restriction (Russel *et al.*, 1968; Thornton *et al.*, 1979; Jopson, 1993). Although at the start of the experiment there were significant differences in the weights of total fat, carcass muscle and visceral lean between the lines, there were no random time x line effects *per se* over the weight loss and realimentation period for any of the components measured in the present study. This indicates that for mature sheep the priorities for and magnitude of the mobilisation of tissue during the weight loss and accretion of tissues during the realimentation were controlled by the relative rate of tissue loss (treatment) and sex of the individual animal, rather than the absolute energy store prior to the weight loss phase.

Jopson *et al.* (1996) indicated that the rate of lean mobilisation in entire fallow bucks during the rut was proportional to the initial weight of fat prior to the weight loss phase. That is that lean conservation was greatest in those bucks that had higher levels of body energy at the start of the fast. This contrasts with the present results, where there was no effect of line (an indirect measure of total body energy) on the weight loss patterns of

either the ewes or the rams during the feed restriction period. The absence of a line effect in the present experiment also contrasts with the results of Rao and McCracken (1991) who showed that pigs that have a higher potential for lean growth are more affected by energy restriction. As the sheep in the present experiment were mature, the potential for lean accretion would be low for both the control and fat lines, resulting in similar responses to the restriction and realimentation phases.

Several studies have shown that the feed required to maintain weight declines over an extended period of time (Ledger and Sayers, 1977; Foot and Tulloh, 1977; Ryan and Williams, 1990; Afonso, 1992; Kolstad and Vangen, 1995). This implies that the weight of body tissue that should be maintained if animals are fed at a constant feed intake, will increase over time as a result of the decline in maintenance requirements as the animal adapts to the period of restricted feeding (Ledger and Sayers, 1977). The results of the present experiment show the reverse, with ewes fed at maintenance showing no significant change in empty body weight and rams showing a marked decline in empty body weight over the experimental period. These results indicate that the maintenance requirements of mature ewes does not vary over time, if expressed on a body weight basis, whilst for rams the decline in weight indicates that the maintenance requirements per unit of empty body weight increased during the final period of the experiment.

Studies that have forced immature ruminants to maintain weight by restricted feeding clearly show that the relative proportion of visceral organs decreases with time (Foot and Tulloh, 1977; Murray and Slezacek, 1988b; Ryans and Williams, 1990). The body weight of those immature animals was maintained by an increase in carcass weight and particularly fat weight (Notter *et al.*, 1983; Aziz and Murray, 1987; Jopson *et al.*, 1994). The only significant change in the body components of sheep fed at a constant level for the experimental period was in carcass muscle. In contrast to the results observed in immature animals where no real change in muscle weight was recorded (Aziz and Murray, 1987; Murray and Slezacek, 1988a; Ryan and Williams, 1990), the mature rams in the present experiment mobilised significant levels of carcass muscle in the latter period of the experiment. This loss of carcass muscle represented the majority of empty body

weight loss that has been described previously. The ewes also mobilised carcass muscle, although the rate of decline was less than that for the rams.

It is possible that the reduction in carcass muscle in the maintenance fed sheep, observed in the latter period of the experiment (late summer) was not a result of maintenance feeding *per se*, but was an effect of a seasonal influence on the priorities for fat and lean deposition or depletion. A seasonal reduction in lean weight after adjusting for changes in empty body weight has been shown in immature sheep (Ball *et al.*, 1996), mature fallow deer females (Weber and Thompson, 1995) and mature beef cows (Laurenz *et al.*, 1992).

There were no significant changes in visceral lean weight for either sex fed at maintenance. The reduction in visceral organs noted in immature animals maintaining weight has been implicated as a possible metabolic adaptation which allows animals to reduce maintenance requirements (Koong *et al.*, 1985; Ferrell, 1988; Iason and Manetcom, 1993). Freetly *et al.* (1995b) showed that the oxygen consumption of visceral organs required between 21-42 days to equilibrate, when weight stasis was applied to mature ewes. In the present experiment, the first estimate of visceral weight was made after a minimum period of 42 days at maintenance feeding, so it is possible that a reduction in visceral organ mass and probably metabolic activity had occurred prior to the first scan.

Although there were no significant changes in total fat weight of mature sheep fed a maintenance, there were minor changes over time which resulted in periods where the rams differed in fat weight to the ewes. The present experiment did not show any apparent oscillations in the spline analysis of total fat weight, however evidence from other experiments would suggest that mature animals experience seasonal influences on body components (Laurenz *et al.*, 1992; Weber, 1996). The apparent difference between mature sheep (where fat is maintained) and immature sheep (where there is an accretion of carcass fat; Notter *et al.*, 1983; Afonso, 1992; Vanger and Thompson, 1992) may indicate that for mature sheep the impetus for growth has been reduced, whilst in immature animals there are still priorities for growth. The result in mature sheep does not concur with the

hypothesis of Black (1974) who stated that a period of maintenance feeding will result in increased carcass lean and decreased carcass fat. Although Yambayamba *et al.* (1996b) showed that there was a loss of subcutaneous fat tissue and that carcass muscle was maintained in beef heifers that were fed to maintain weight for a period of 92 days

It is interesting to note that the response in immature pigs opposes both the observed results in mature and generally for immature ruminants. Recently, Luiting *et al.* (1995) and Kolstad and Vangen (1995) has shown that there was a marked loss in total fat and a gain in carcass muscle in pigs that were maintaining weight. Clearly further research is required to determine whether true differences exist in the response of mature and immature animals to a period of maintenance feeding.

Overall, the comparison of sheep fed at 80% of maintenance to sheep fed at 60% within sexes for EBW shows that the rate of weight loss depends on the level of feeding below maintenance. A higher level of restriction will result in a higher rate and a greater absolute loss of weight over a fixed time period. A comparison of the sexes fed at 80% of maintenance indicates two different mechanisms of adaptation of mature ewes and rams to a mild weight loss period. For the ewes the conservation of body tissue in the early phase of the restriction suggests that the metabolic activity of the total body must have been reduced rather than mobilising body tissues to service the energy deficit.

A number of authors have shown that sheep losing weight reduce maintenance requirements (Graham and Searle, 1979; Gingens *et al.*, 1980; Ryan *et al.*, 1993a) and this is primarily through a reduction in the mass of metabolically active visceral organs (Koong *et al.*, 1985; Ferrell, 1988). The present study showed that there was no change in the mass of the visceral organs for ewes fed at 80% of maintenance it is possible that the energy requirements per unit of these tissues declined during the period of constant feeding prior to the start of the experiment. Freetly *et al.* (1995b) showed in chronically undernourished mature ewes that there was a decline in metabolic requirements during the initial 21-42 days of the restriction. As the length of time of the restriction increased it appeared that the metabolic adaptation was unable to completely match the requirements of the body to

the level supplied from the feed resulting in a loss of empty body weight towards the end of the restriction.

In contrast, the rams mobilised body tissue at the start of the restriction phase with the rate of mobilisation declining as the time period of the restriction increased. This suggests that the loss of body tissues experienced by the rams in the early phase reduced the requirements, so by the end of the restriction they were approaching an equilibrium body weight respective to the level of intake below maintenance.

The sheep fed at 60% of maintenance mobilised body tissue at a continuous rate for the length of the experiment. As with the sheep fed at 80% of maintenance, rams lost proportionally more tissue from the body than the ewes. This suggests that either the metabolic adaptation to the restriction was greater in the ewes, or that a higher proportion of the energy dense fat tissues were mobilised by the ewes than by the rams, which is reflected in the lower weight loss.

The apparent effect of severity of restriction on the patterns of weight loss in mature sheep agrees, in general, with the patterns of weight loss in immature sheep fed at different levels of restriction (Butler-Hogg, 1984; Carstens, 1995). Kabbali *et al.* (1992b) showed that there was no difference in the weight loss patterns between lambs fed at 200g/day compared to lambs fed at 100g/day during the initial reduction in liveweight from 25-20kg, although the lambs fed at 200g showed no further reduction in liveweight indicating that a nadir had been reached at this point.

When the previously restricted sheep were returned to the original levels of feed required for weight maintenance at the start of the experiment, both the 80% and 60% groups of rams gained EBW, whilst the ewes had no overall gain in EBW and remained at the same weight as that at the end of the restriction. For sheep fed at 80% of maintenance it appears that the ability to regain tissue was inhibited, with the empty body weights at the end of the experiment being less than that at the start for both sexes. It is apparent that the sheep that were restricted at 60% of maintenance were severely affected by the restriction

and failed to regain, during the realimentation period, the majority of the tissue lost during the restriction. This suggests that imposing a severe restriction on mature sheep changes the metabolism of the body significantly, resulting in a different equilibrium weight being obtained for these sheep. Ryan (1990), indicated that animals at, or near maturity, that experience a period of severe weight loss may not compensate fully when fed *ad libitum*, if compensation is expressed on a live weight basis. A similar conclusion was reached by Butler-Hogg and Tulloh (1982).

Overall, the inability of the two restriction groups to compensate, contrast with studies on weight loss and regain in immature animals. As the severity of the restriction increased the rate of compensation increased in growing lambs (Greeff *et al.*, 1986b) and steers (Carstens *et al.*, 1991). This suggests that given a sufficient length of time the phenomena of compensatory growth provides the ability for previously restricted immature animals to attain similar weights as normally grown animals (Thorton *et al.*, 1979; Butler-Hogg and Turgeon, 1982; O'Donovan, 1984; Ryan, 1990; Carstens, 1995). However Ryan *et al.* (1993b) indicated that increased restriction resulted in reduced capacity of the animal to show compensation, which agrees with the results in the present experiment. It must however be stated that in the majority of studies on compensatory growth, the period of realimentation usually involves *ad libitum* feeding, whilst in the present study the sheep were restricted to previous levels of maintenance.

Taylor and Young (1968) showed that for cattle fed to increasing equilibrium weights there was a consistency between the equilibrium weight obtained and the higher equilibrium feed intake. The slope of this relationship T_0 (Taylor Time Constant; Parks, 1992) has been generally assumed to be constant. The results of the present experiment indicates that a period of restricted feeding and then subsequent re-feeding at the original level forces a mature animal to deviate from the equilibrium situation that Taylor and Turner (1968) established. This indicates that the utilisation of feed for equilibrium weight maintenance can be altered by a nutritionally induced change in the physiological state of the animal. The sheep that were restricted to 80% of maintenance clearly reduced maintenance requirements in proportion to body mass as the level of weight loss should

have been higher than that recorded. This reduction in maintenance requirements was noted by Wolkers *et al.* (1994b) where the weight reduction in deer was far less than that predicted to occur during a feed restriction period. Wester *et al.* (1995) showed from a detailed study on the oxygen consumption of the visceral organs, that the metabolic activity as a function of mass was not constant in lambs losing weight.

3.4.2 Changes in body components during feed restriction

There are numerous experiments that have attempted to monitor the mobilisation of specific components of the body during weight loss induced by a period of restricted feeding through serial slaughter or by *in vivo* estimates obtained from radioisotope dilution techniques. The majority of these studies have shown that there is a distinct hierarchy in the priorities for tissue mobilisation (Searle *et al.*, 1979; Butler-Hogg, 1984; Aziz *et al.*, 1992; Kamalzadeh, 1996). The ability to accurately measure the weights of body components over time using the CAT-scanner provides a unique opportunity to detect the differential loss and regain of fat, muscle and viscera which has not been possible through serial slaughter designs

In the present experiment there were clear effects of the severity of the restriction and the sex of the animal on the priorities for mobilisation of the tissues of the body. Searle *et al.* (1979), Butler-Hogg (1984) and Aziz *et al.* (1992) all showed that the rate of protein loss was greater in the early phase of the restriction and that as the length of the restriction increased, the rate declined. This appears to be the pattern for rams fed at 80% of maintenance and for both sexes fed at 60% of maintenance, where the loss of carcass muscle was higher during the early phase and was approaching zero at the end of the restriction.

In energetic terms the mobilisation of carcass muscle represented only a minor contribution to the total energy supplied by the catabolism of body tissues during the weight loss period (approximately 7% for the sheep fed at 60% of maintenance). Adamczewski *et al.* (1987) suggested that the loss of protein in the ruminant may be more

a function of protein output from the rumen during the feed deficit. However, Chowdhury *et al.* (1995) indicated that maintenance of lean tissue is possible, via the mobilisation of fat tissue, during weight loss given that the dietary protein supply of the animal is adequate. This indicates that the output of protein from the rumen during weight loss may influence the pattern of tissue mobilisation.

Wolkers *et al.* (1994a,b) put forward the hypothesis that muscle degradation during weight loss enabled deer to protect the body from ketosis that arises from the formation of ketone bodies during lipolysis. Claus and Weiler (1994) indicated that the conservation of lean tissue may be possible due to the anticatabolic function of androgens, particularly in the male. Both Hart *et al.* (1985) and Waghorn *et al.* (1987) indicated that the elevated levels of growth hormone present in sheep during weight loss promoted lipolysis which enabled body protein (particularly in the carcass muscle) to be conserved. Recently Adams *et al.* (1996a) showed that ewes immunised against GHRH had the same relative rate of liveweight loss during weight loss despite growth hormone and IGF-1 levels being appreciably lower. In contrast, Price (1977) considered that the contribution of mobilisation of carcass muscle to the energy balance occurs passively as a consequence of the primary functional role, rather than as an active response to an energy deficit. Overall, this protein sparing mechanism, that is brought into action in undernourished animals may be responsible for the higher efficiency of growth (k_p) that has been noted in compensating animals (Gingens *et al.*, 1980).

In general, the greatest absolute loss of tissue occurred in total fat for all treatment groups fed at below maintenance. This result agrees with the results of Little and Sandland (1975), Kabbali *et al.* (1992b) and Kamalzadeh (1996). The relative loss of fat from the carcass was higher than that for muscle (protein and water) which agrees with the results of Ryan *et al.* (1993b) and contrasts with the results of Kamalzadeh (1996). It is clear that the stage of maturity of the animal at the time at which the restriction occurs influences the relative proportions of fat and muscle lost from the body.

Both sexes fed at 80% of maintenance demonstrated the ability to conserve total fat in the early phase of the restriction and mobilised a greater proportion of the total fat in the latter phase of the restriction. Drew *et al.* (1975a) and Aziz *et al.* (1992) also showed a similar pattern in fat mobilisation and indicated that the contribution of energy from fat mobilisation to the net energy deficit will increase as the length of the weight loss period increases.

In contrast the rate of fat mobilisation was high for the sheep fed at 60%. Butler-Hogg (1984) showed that sheep which lost weight slowly did not extensively mobilise fat depots when compared to sheep that were losing weight at a higher rate. This appears to be confirmed by the present results which contradict the hypothesis of Black (1974), who suggested that the rate of weight loss (severity of the restriction) did have a marked effect on the pattern of fat and lean loss. He proposed that a higher rate of weight loss would result in a greater loss in lean and a lower rate of loss would result in a greater loss of fat.

Dunshea and Bell (1988) indicated that fat loss during chronic undernutrition in goats resulted from a reduction in lipogenesis and intracellular NEFA rather than increased lipolysis. This was confirmed in sheep by the results of Petterson *et al.* (1994). Hood and Thorton (1979), showed that the loss of lipid reserves of mature sheep resulted from decreased cell size rather than a reduction in adipose tissue cell number. As chronically undernourished animals have the ability to mobilise fat stores by mechanisms other than increased lipolysis this enables the animals to reduce the formation of ketone bodies and avoid ketosis.

Jopson *et al.* (1996) showed that there was a preferential conservation of carcass lean by entire males when compared to castrates. In contrast, the present experiment showed that mature males appeared to mobilise greater levels of both fat and carcass muscle when restricted to the same level of below maintenance feeding compared to females. Observations in young bulls undergoing weight loss showed a decline in fat during the restriction period, but protein levels remained stable (Henricks *et al.*, 1994).

Thus the level of conservation of muscle mass may depend on the maturity of the male at the time when the restriction is applied.

Although the females had higher levels of energy stores in the body prior to the weight loss phase, it appears that reduction in weight loss is achieved by reducing metabolic demands rather than having a higher level of mobilisation of the energy dense tissues. This evidence suggests that differences in rates of weight loss that have been observed between entire males and either castrates or females may not be due to a greater capacity to mobilise energy dense fat depots (Russel *et al.*, 1968; Price, 1975).

There was a marked affect of the severity of the restriction on the rate of visceral lean mobilisation. Meyer and Clawson (1964), Winter *et al.* (1976), Thorton *et al.* (1979) and Ryan *et al.* (1993b) have all shown that visceral organs are severely depleted in the early phase of weight loss in immature sheep. This is consistent with the results for the sheep fed at 60% of maintenance, where there was a 20% loss in visceral weight in the initial weight loss period. Thereafter the decline in visceral weight for this group was minimal. In contrast, there was no significant change in the weight of the visceral organs of the sheep fed at 80% of maintenance. This difference suggests that there is a threshold point for the severity of the restriction which either results in the loss or preservation of visceral organ mass during weight loss.

3.4.3 Changes in body components during realimentation

The realimentation phase of the present experiment was dominated by the inability of the sheep to regain tissue that was lost during the restriction phase. Clearly the period of weight loss resulted in a permanent change in the mass of carcass muscle that was initially maintained by the maintenance level of feeding. This is in dramatic contrast to the results obtained in immature animals that experience a period of weight loss and subsequent period of realimentation. Kamalzadeh (1996) clearly showed that the rate of lean accretion in restricted immature lambs was greater during a realimentation period at

ad libitum (0.184 kg protein / kg carcass weight) relative to controls (0.175 kg protein / kg carcass weight).

Butler-Hogg (1984), Wright and Russel, (1991) and Ryan *et al.* (1993b) all indicated that two phases of tissue deposition occurred during realimentation. Initially there was an increase in protein deposition that was primarily associated with replenishment of visceral and gut tissue. In the present experiment only the sheep fed at 60% of maintenance experienced a significant change in visceral weight during the restriction period, which was subsequently recovered during the realimentation period. This result is in agreement with Winter *et al.* (1976) who noted that the proportions of the non-carcass components formed a decreased component of EBW during weight loss and increased in relation to EBW during realimentation.

The ability of immature animals to increase the rate of lean deposition during the realimentation period (Marias *et al.*, 1991a; Kabbali *et al.*, 1992b; Kamalzadeh, 1996), may be reliant on a reduction in the rate of turnover of lean tissue during the restriction phase. Kreienbring *et al.* (1994) showed that the compensation of lean tissue in immature rats was due to an increased rate of synthesis of protein. Several studies have shown that the rate of protein turnover decreases with age (Waghorn and Wolf, 1984; Webster, 1989; Claus and Weiler, 1994). The inability of mature sheep to regain lost carcass muscle in the present experiment results from a reduced capacity for protein synthesis to be stimulated above those levels for maintenance of the weight of carcass muscle. Interestingly Bikker *et al.* (1996) showed that pigs who were fed at restricted levels (1.7 times maintenance) during the realimentation phase, had higher rates of fat deposition and lower rates of lean deposition relative to control animals. As the level of feeding during realimentation increased, fat deposition rates declined and lean deposition rates increased. It might be that level of feeding during the realimentation period has an affect on the relative deposition rates of body tissues and that this may be a cause of the variation in published results for body development in compensating animals.

The second phase of the realimentation period, that was identified, was an increase in fat deposition after repletion of the protein components (Ryan, 1990; Wright and Russel, 1991; Carstens, 1995). It appears that the rams have the ability to regain a portion of the fat tissue that was lost. The severity of the restriction influenced the magnitude of the regain of fat weight, with the rams fed at 80% recovering all lost fat tissue, whilst the rams fed at 60% only regained 25% of the lost fat tissue. The ewes however, did not demonstrate the ability to regain any of the fat tissue that was lost during the restriction period. This is in contrast with the results of Yambayamba and Price (1991) who provided evidence that a higher priority may be given to fat growth in the realimentation period in growing females.

The stimulation of fat deposition in realimentating animals after a period of weight loss has been observed by Meyer and Clawson, 1964, Allden, (1970), Greeff *et al.* (1986b), Yambayamba and Price (1991) and Iason and Mantecom, (1993). Greeff *et al.* (1986b) indicated that improved efficiency resulting from a high degree of restriction leads to higher fat deposition and a possible shift in metabolism that favours fat tissue development. They showed that severely restricted animals are fatter after a period of realimentation as a result of the shift to a fat based metabolism. Adams *et al.* (1996b) showed that fat deposition was elevated in ewes immunised against GHRH during a period of realimentation after weight loss. They indicated that the lower GH, IGF-1 and prolactin levels in the immunised ewes favoured the deposition of fat tissue. Thus it is possible that the result obtained for this experiment represents a reduced sensitivity to GH, IGF-1 or prolactin in mature animals.

Schadereit *et al.* (1995), indicated that realimentated rats favoured the deposition of fat at the expense of protein. They suggested that there were two possible explanations for the higher fat gain. In their study, there was a causal relationship between delayed weight gain in the liver and a slower protein synthesis, which they speculated provided more energy for fat synthesis as a result of lower energy requirements needed for protein synthesis. Schaderiet *et al.* (1995), also speculated that the higher fat gain is a function of age and therefore the restricted rats have to replace fat in the adipose tissues that have been

depleted during restriction. Rozen *et al.* (1994) showed that the practice of successive restriction and refeeding in rats did not result in resistance to weight loss but rather a defect in the utilisation of energy intake that facilitated the development of obesity. It is interesting to speculate whether a similar metabolic shift occurs in mature ruminants.

The results obtained for mature animals clearly are different to those from the literature on compensatory growth in immature animals. The studies of Fox *et al.* (1972), Gingens *et al.* (1980), Wright and Russel (1991) and Carstens (1995), all showed that the phenomena of compensatory growth allows previously restricted animals to obtain a comparable body composition to animals that have experienced normal growth. The results obtained for mature animals suggests that a period of feed restriction at maturity can permanently alter the equilibrium body composition of animals that are fed at maintenance levels. Broekhuizen *et al.* (1994), stated that the intervention of a period of feed restriction must act on long term tissue turnover and repair rates and it appears that this disruption in mature animals causes an effective change to a new equilibrium weight and body composition.

3.4.4 Fat partitioning

In the present study, the majority of change in total fat weight that occurs during the restriction and realimentation period is a result of mobilisation and deposition of adipose tissue within the carcass depots. Little and Sandland (1975) showed that the most labile fat depot was the subcutaneous depot in immature sheep losing weight. Jopson *et al.* (1996) recorded extreme rates of fat mobilisation, with external fat depots mobilised first in comparison to internal fat depots. The results for the animals fed at 60% of maintenance are in agreement with both of these studies. They contrast however with Kirton and Barton (1958a) and Hight and Barton (1965), who were unable to demonstrate a significant reduction in subcutaneous fat, when aged ewes (4 to 6 years old) were kept at low levels of feeding for either short or long periods.

Butler-Hogg (1984) indicated that sheep that were losing weight slowly, suffered little loss in either carcass or internal (visceral) fat depots. This is clearly evident for sheep

that were fed at 80% of maintenance where there was only a minor loss in carcass fat (after an initial gain) during the restriction period and a regain of carcass fat during the realimentation phase.

In the results of Butler-Hogg (1984) and Jopson (1993) there was a linear time by treatment effect on internal fat depots suggesting that mature animals preferentially mobilise this tissue during a period of weight loss. This result also agrees with studies in both immature and mature sheep undergoing periods of weight loss, where significant changes in internal fat levels have been noted (Robinson, 1948; Seebeck, 1973; Thorton *et al.*, 1979; Kabbali *et al.*, 1992b). In the studies of Hodge and Star (1984), Hayden *et al.* (1993), Kabbali *et al.* (1992a) and Kabbali *et al.* (1992b) young animals lost body tissues during a period of weight loss, during realimentation all tissues were regained except for internal fat which remained at depleted levels.

Hayden *et al.* (1993) put forward the hypothesis that the restricted phase had a greater effect on the earlier maturing internal fat depots of immature animals when compared to the later maturing subcutaneous and visible intramuscular fat tissues which undergo lipid accumulation during the later stages of compensatory growth. The results of the present experiment indicate that the priorities for replenishing fat tissues are directed towards the carcass fat depots. Adams *et al.* (1996b) showed that ewes immunised against GHRH had higher relative rates of fat deposition in the internal depots during the realimentation period. Thus it appears as though the partitioning effect present in this experiment was not a result of lower GH, IGF-1 or prolactin levels.

In the present experiment it appears as though internal fat continued to be mobilised, whilst there was regain of carcass fat and muscle by the groups that had experienced a period of restricted feeding. Interestingly the sheep fed at maintenance also experienced a minor decline in internal fat. This contrasts with the results of Murray and Slezacek (1988b) who showed there was no effect on internal fat depots during a period of weight stasis in wethers. However, Afonso (1992) noted a curvilinear decrease in the internal fat weight of immature sheep fed to maintain liveweight, whilst Kolstad and

Vangen (1995) observed a decrease in the internal fat depots of pigs fed to maintain liveweight. A recent study of Yambayamba *et al.* (1996b) indicated that the internal fat depot of heifers that were restricted to maintain weight decreased slowly and then increased slowly during a subsequent realimentation period. They suggested a prolonged lag phase in the response of internal fat depots to nutritional manipulation.

3.5 Conclusion

Palsson (1955), in a review of the literature of the affects of plane of nutrition on body composition, concluded that animals that are subjected to a period of sub-maintenance feeding utilise tissues from body regions in the reverse order of their maturity. That is fat followed by muscle then bone in order to supply energy and protein for maintenance. The present experiment shows that this conclusion is not applicable for mature animals. Initially carcass muscle is mobilised to support the nutrient deficit of the restricted feed period. As carcass muscle reserves are depleted, fat mobilisation occurs in the later phase of the restriction.

Clearly, the level of the restriction and the sex of the animal influenced the priorities for tissue loss. A more severe rate of weight loss promoted the depletion of visceral lean and increased the relative loss of total fat during the early phase of the restriction. From this experiment, it is evident that indices based on linear or visual estimates of body fatness (that is fat depth or body condition score), that assess body energy reserves in mature sheep during a period of weight loss, may not reflect the true impact that weight loss has on body tissue catabolism. In this experiment, there was no effect of initial body energy reserves, as tested by the comparison of the lines selected for backfat thickness, on the mobilisation and accretion of body tissues during the restriction and realimentation periods, respectively.

Robinson (1948) suggested that mature ewes gained and lost both muscle and fat on similar paths during super-maintenance and sub-maintenance feeding. Results from the present experiment show this is not the case when mature animals are fed at sub-maintenance levels and then refed at maintenance. The endocrine and metabolic shifts that occurred during the weight loss phase altered the animals priorities for tissue deposition resulting in a shift towards fat deposition. The phase of fat deposition that was apparent in the realimentation phase may reflect a situation where the propensity for muscle deposition in the mature animal is virtually zero and that any excess nutrients above that required for maintenance are utilised for fat deposition.

The marked difference between the weight and composition of the body of those sheep that had been fed at levels below equilibrium and returned to equilibrium feeding levels was unexpected. Further analysis of the relationship between equilibrium feeding levels and equilibrium weight in mature animals is required.

Chapter 4

Changes in maintenance efficiency of mature sheep fed at different levels of feed intake.

4.1 Introduction

Brody (1945), Klieber (1975) and more recently Baldwin (1995) indicated, the energy dynamics of an individual animal follows the first law of thermodynamics. The law states that “*energy can be transformed from one form to another but cannot be either created nor destroyed*”. From this law has developed one of the most widely used techniques for estimating maintenance energy requirements (Me_m), where Me_m can be calculated as the difference between measured levels of total metabolisable energy intake and the energy retained or deposited in the growth of body tissue (ARC, 1980).

$$Me_m = \text{Energy intake (MEI)} - \text{retained energy (RE)}$$

Obviously this procedure relies on an accurate estimate of the measured components. That is, of energy supplied by the diet and the energy change within the body that occurs over a period where the feed supplied to the animal is measured. A number of experiments have attempted to define retained energy as either linear or non-linear functions of the rate of change in liveweight, body weight or of the components of fat and protein multiplied by energy content of the tissue (Ferrell *et al.*, 1976; Roux *et al.*, 1982; Olthoff *et al.*, 1989; Tess and Greer, 1990; Bishop, 1992). The more robust estimates have been obtained by procedures where changes in protein (lean) and fat have been measured using direct methods of serial slaughter, or indirectly by techniques such as specific gravity, ultrasonics or isotope dilution techniques (Panaretto, 1964; Graham, 1967; Garrett and Hinman, 1969; Russel and Wright, 1982; Kempster, *et al.*, 1982). However these estimates are often inaccurate due to either sampling problems associated with allocation of animals to slaughter groups, or with extrapolation of estimates of total body water to weights of body fat and lean.

The ability to obtain accurate repeated estimates of body components in the live animal, through the use of the CAT-scanner (Thompson and Kinghorn, 1992; Afonso, 1992), provides the opportunity to calculate changes in the energy content of the body of an animal over a period where intake is controlled. Enting *et al.* (1990), Jopson *et al.* (1994), Luiting *et al.* (1995) and Kolstad and Vangen (1995;1996) have used this technique to estimate the “true” costs for maintenance by accounting for changes in the energy content of the body for immature and mature sheep and pigs during a period of maintenance feeding. The additional advantage of this procedure is that differences in the retained energy content of the body can be calculated repeatedly for individual animals over several periods where feed intake levels can be varied.

The experimental design of Taylor and Young (1968) initiated that equilibrium maintenance requirements can be estimated from the relationship between the equilibrium weight and the equilibrium feed intake of the animal. Taylor *et al.* (1981) showed that this relationship, known as the efficiency of maintenance of equilibrium weight (T_0), was constant for an individual animal. Taylor and Turner (1968) showed that T_0 was constant in immature cattle that were fed at increasing equilibrium levels of feed intake, but noted that the relationship between A (weight) and q (weekly feed intake) may not hold for animals that have lost weight. In contrast, Parks (1982) suggested, from the use of the Taylor diagonal in his growth phase plan, that an animal fed at below maintenance (q') will lose weight until a equilibrium weight of $A' = T_0q'$. There are studies that have used the design of Taylor and Turner (1968) to estimate maintenance requirements of immature animals after correcting for changes in the energy content of the body during the equilibrium period (Afonso, 1992; Kolstad and Vangen, 1995). However in the previous chapter, (Chapter 3) it was shown that the final body composition of a mature sheep, that had undergone periods of weight loss and realimentation, differed to that measured prior to the weight loss period. To date, no study has attempted to show whether the concept of T_0 can be applied to animals that have lost and subsequently recovered weight.

The exponent of the relationship between maintenance requirements (as estimated from fasting heat production) and body weight for mature animals was originally proposed

by Klieber (1975) to be 0.73. For immature animals Thonney *et al.* (1976) showed that the exponent can differ from 0.4 through to 0.84. They indicated that heat production (and therefore estimates for maintenance) can be adequately predicted in most cases by a linear relationship to body weight with a non-zero intercept. Taylor and Young (1968) showed that the coefficient of the relationship between log equilibrium feed intake and log equilibrium weight did not differ from unity, but differed significantly from 0.75. Vercoe and Frish (1977), Gettys *et al.* (1987) and Carstens *et al.* (1989) also showed that the relationship between estimates for maintenance requirements and body weight did not differ from unity. In order to examine the concept of T_0 using the present experimental results estimates for maintenance requirements for individual animals were scaled for changes in empty body weight raised to the power of one.

A number of workers have suggested that lean body mass may be more appropriate for comparing the differences in maintenance requirements between breeds and between animals at different stages of maturity (Graham, 1967; Pullar and Webster, 1977; Webster, 1981; Tess *et al.*, 1984). It may be that differences in the maintenance requirements that have been recorded in sheep that are undergoing weight loss or weight gain are related to differences in body protein mass (Graham, 1967; Ortigues *et al.*, 1993). This question was examined by scaling the estimate for maintenance requirements of an individual sheep for differences in the weight of body lean at the start of each period (Chapter 3). As the sheep had been shown to change in the relative proportions of body tissues during the course of feed restriction and subsequent realimentation (Chapter 3), the question arose as to whether the requirements for energy retention in the body as a function of the energy present in the body changed over the experimental period. In order to test this hypothesis, estimates for maintenance requirements over time were scaled for differences in the energy content of the total body at the end of each period.

4.1.1 Null Hypothesis

That the relationship between the equilibrium weight and feed intake in mature sheep that are feed restricted and then refed to original levels of liveweight maintenance is constant after adjusting for differences in the retained energy content of the body.

4.2 Materials and Methods

The experimental design for the data used has been described in detail in chapter 3. Briefly estimates of body component weights were obtained for 24 individual sheep (12 rams and 12 ewes from two lines selected for i) increased backfat thickness and ii) a randomly bred control line; Fennessy *et al.*, 1987) that had been fed at either a constant feed level (treatment 1 100%; n=8), or at levels below maintenance (80% and 60%; n=8 respectively) for a period of 15 weeks and then at original levels for body weight maintenance for a realimentation period of 15 weeks (treatments 2 and 3). The experimental design is shown in figure 3.2.1.

Changes in total body energy were calculated from differences that occurred in the energy content of total fat, carcass muscle and visceral lean for individual sheep between the consecutive scanning periods. The weight differences for the lean components (carcass muscle and visceral lean) were multiplied by 4.74 MJ/kg assuming that the lean depots contained 20% protein (Webster, 1980; Moloney *et al.*, 1995) which had a standard energy value of 23.7MJ/kg (Webster, 1977). The weight differences for total fat were multiplied by 31.44 MJ/kg assuming that estimate of fat in the live animal contained 80% chemical fat (Thompson, 1983) that has a standard energy content of 39.3 MJ/kg (Webster, 1977, 1980). As the changes in the energy content of the tissues occurred during periods of weight loss and gain, the energy changes were corrected for an efficiency of protein gain (k_p) of 0.20, an efficiency of fat gain (k_f) of 0.70 and an efficiency of energy utilisation of tissue loss (k_l) of 0.80 (for both fat and lean depots) (Rattary and Joyce, 1976; Gengens *et al.*, 1980; Geay, 1984). The metabolisable energy supplied by the feed was calculated based on the assumption that the feed contained 10.2 MJ ME/kg as measured from *in vivo* digestibility estimates (Thompson, 1983; Table 3.1)

Maintenance requirements for each sheep over the eight periods were calculated from the equation

$$ME_m = MEI - (REC + REF + RE_V)$$

where ME_m = maintenance energy requirement (MJ ME/day)

MEI = metabolisable energy (MJ ME/day)

REC = retained energy for carcass lean (MJ/day)

REF = retained energy for total fat (MJ/day)

RE_V = retained energy for visceral lean (MJ/day).

These estimates for ME_m were scaled for differences in the empty body weights (Me_mKg) of the sheep, for differences in total body lean (Me_mLn) or for differences in the energy content of the body (Me_mEn) at the start of the each period. The parameter Me_mKg is defined as the feed requirements (MJ ME) required to maintain a kilogram of empty body per day, Me_mLn is the feed required to maintain a kilogram of total lean per day and Me_mEn is defined as the feed requirements (MJ ME) required to maintain a megajoule of body energy per day.

Although Me_mKg (MJ ME/kg day⁻¹), Me_mLn (MJ ME/kg day⁻¹) and Me_mEn (MJ ME/MJ day⁻¹) are derived variables, they are still repeated estimates that have been obtained from each animal over time. Variograms (Diggle, 1990) showed that the error structure for the repeated estimates were dominated by random effects (animal). The analysis used the techniques of Cullis *et al.* (1996) and the accompanying S-plus functions to incorporate cubic smoothing splines into a random effects model. A full model initially fitted was

$$Y_{ijkl} = \mu + T_i + S_j + L_k + TS_{ij} + TL_{ik} + SL_{jk} + TSL_{ijk} + D_l + F(D_l) + R(D_l) + TD_{il} + T_iF(D_l) + T_iR(D_l) + DS_{jl} + S_jF(D_l) + S_jR(D_l) + DTS_{ijl} + TS_{ij}F(D_l) + TS_{ij}R(D_l) + Animal_{ijk}$$

where

Y_{ijkl} is the response for treatment i , sex j , line k and day l .

μ is the overall mean

T_i is the i^{th} treatment effect

S_j is the j^{th} sex effect

L_k is the k^{th} line effect

D_l is the linear time term that was scaled to centred mean by (time-mean(time))/ $\sqrt{\text{variance of time}}$

$F(D_l)$ is the non-linear component of the model that is assumed to have the form of a cubic spline

$R(D_i)$ is the random time effect that reflects the lack of fit from the corresponding fitted cubic smoothing spline

$Animal_{ijk}$ is the random animal effect

Variance components (random effects; interactions with either spline time or random time) were then tested for significance by successively dropping their terms from the model and assessing the change in the log-likelihood statistic. When the appropriate variance component model for the two parameters had been selected, the significance of the fixed effects ($P < 0.05$) was determined using a two-tailed t-test. The significant effects, as well as their non-significant lower order terms, were then included in the final model for each derived parameter. Treatment, line and sex were fitted using Helmert contrasts.

The deviation away from the initial estimate of Me_m at the start of the experiment for each parameter over the experiment period, rather than the absolute mean Me_m tested the hypothesis that T_0 remains constant over time. The components of the fixed effects and the random terms relevant to the slope (“shape”) are shown in graphs as a deviation from zero (initial estimate of Me_m). The respective 95% confidence intervals for each spline were calculated as follows

$$\hat{y} = x\hat{\beta} + Z\hat{u}$$

$$\text{var}(\hat{y}) = x \text{var}(\hat{\beta}) x^T + Z \text{var}(\hat{u}) Z^T + 2 * \text{cov}(x\hat{\beta} + Z\hat{u})$$

where

x is the design matrix of the fixed effects

Z is the design matrix of the random effects

$\hat{\beta}$ is the estimated fixed effects

\hat{u} is the estimated random effects

4.3 Results

The t-value and the significance of the t value for the fixed effects of the final models for Me_mKg , Me_mLn and Me_mEn are presented in Table 4.3.1. The significant random effects from the final models for Me_mKg , Me_mLn and Me_mEn are presented in Table 4.3.2.

Table 4.3.1 Significance and t-values for the overall mean (μ), deviations for fixed effects from the overall mean and deviations for the linear time effects for the derived variables of maintenance requirements per kilogram of weight (Me_mKg), maintenance requirements per kilogram of total lean (Me_mLn) and maintenance requirements per MJ of body energy (Me_mEn).

| Parameter | Mean | Fixed Effects | Time effects | t-value |
|----------------------------|--------|---------------|--------------|-----------|
| Me_mKg | | | | |
| Mean (MJ ME/Kg/Day) | 0.192 | | | 24.290*** |
| | | Treat1 | | -1.761** |
| | | Treat2 | | -3.050*** |
| | | Sex | | -1.946** |
| | | | time | 1.978*** |
| | | | Treat1*time | 3.752*** |
| | | | Treat2*time | 5.949*** |
| Me_mLn | | | | |
| Mean (MJ ME/kgLn/Day) | 0.323 | | | 146.69*** |
| | | Treat1 | | -6.704*** |
| | | Treat2 | | -7.254*** |
| | | Sex | | 3.623*** |
| | | Line | | 3.278*** |
| | | Treat1*sex | | 1.930** |
| | | Treat2*sex | | -1.552* |
| | | | time | 10.487*** |
| | | | Treat1*time | 2.971*** |
| | | | Treat2*time | 3.937*** |
| | | | Sex*time | 1.871** |
| Me_mEn | | | | |
| Mean (MJ ME/MJ/Day) | 0.0168 | | | 19.462*** |
| | | Treat1 | | 1.162 |
| | | Treat2 | | -1.112 |
| | | Sex | | -6.997*** |
| | | Line | | -2.884*** |
| | | Treat1*sex | | -1.952** |
| | | Treat2*sex | | 1.330* |
| | | Sex*Line | | 1.649* |
| | | | time | -2.473*** |
| | | | Treat1*time | 3.462*** |
| | | | Treat2*time | 5.695*** |
| | | | sex*time | -1.806** |

* P < 0.1

** P < 0.05

*** P < 0.01

The three treatments of 100%, 80% and 60% were fitted as Helmert contrasts as Treat1 and Treat2.

Table 4.3.2 Significant variance components (Random effects) of the final models for the derived parameters of Me_mKg , Me_mLn and Me_mEn .

| Parameter | Me_mKg | Me_mLn | Me_mEn |
|-------------------------------------|----------|----------|----------|
| Random effects | | | |
| Spline time $F(D_i)$ | ✓ | ✓ | |
| Random time $R(D_i)$ | ✓ | | ✓ |
| treatment * Spline time $T_iF(D_i)$ | ✓ | ✓ | |
| sex * spline time $S_jF(D_i)$ | ✓ | | |
| animal | ✓ | ✓ | ✓ |

✓ Indicates significant random effects (variance components) at $P < 0.05$

4.3.1 Deviations over time for Me_mKg

The final model for the feed energy required to maintain a kilogram of body weight (Me_mKg) contained the random terms for treatment*spline time, sex*spline time, sex*random time and animal as shown in Table 4.3.2. The sex effect (± 0.03 MJ ME/Kg day⁻¹) indicated that ewes required 16% less energy to maintain a kilogram of body weight at all stages of the experiment.

As shown in figure 4.3.1, the treatment*spline time indicated that sheep fed at 60% of maintenance had a decline in Me_mKg during the initial stages of the weight loss period, however in the final stages of the weight loss Me_mKg increased so there was no difference between this estimate and that obtained prior to the weight loss phase.. At the end of the realimentation period sheep that were fed at 50% of maintenance during the weight loss period required 15% more feed to maintain a kilogram of empty body weight than that required at the start of the experiment. In contrast although sheep fed at 80% showed a decline in Me_mKg during the early stages of weight loss and an increase during realimentation, the level of feed required to maintain empty body weight was not significantly different from that measured at the start of the experiment. Only small non-significant changes in Me_mKg were noted for the sheep fed at 100% of maintenance as shown in figure 4.3.1.

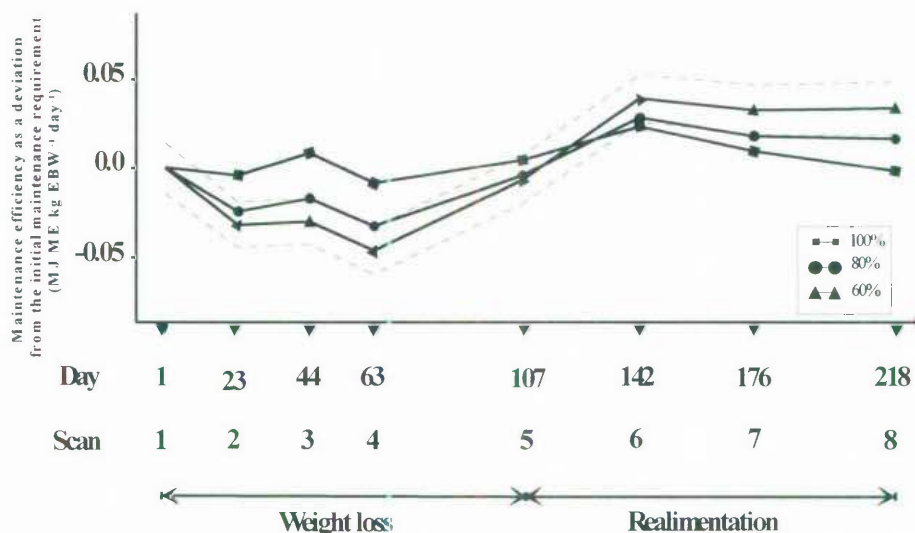


Figure 4.3.1 Deviations for Me_m Kg ($MJ ME / kg day^{-1}$) from initial estimate for the feed required to maintain a kilogram of empty body weight (scaled to zero) for the three treatment groups during the restriction and realimentation phases of the experiment. The dotted lines represents the 95% confidence limits for sheep fed at 60% of maintenance.

4.3.2 Deviation over time for $Me_m Ln$

The final model for the parameter $Me_m Ln$ contained significant terms for treatment*spline time, treatment*linear time and sex*linear time (Tables 4.3.1 and 4.3.2). The sex*linear time effect indicated that after maintenance requirements per kilogram of total body lean were lower at all times for the ewes when compared to the rams. As shown in figure 4.3.2, $Me_m Ln$, although fluctuating, did not significantly change for the sheep fed at maintenance over the entire experimental period. In contrast, $Me_m Ln$ decreased in the sheep that were fed below maintenance during the restriction period, at day 63 (scan4) the maintenance requirements per kilogram of total body lean was 25% lower than that recorded at the start of the experiment. From day 63 to day 105 there was an increase in $Me_m Ln$, however despite this increase at the end of the restriction period, sheep that had lost weight (i.e. the sheep fed at 80% and 60% of maintenance) still had lower maintenance requirements than sheep that had been fed at maintenance. There was no significant difference in $Me_m Ln$ between the 60% and 80% of maintenance fed sheep during the restriction period.

During the realimentation period, $Me_m Ln$ increased for the sheep that had been previously restricted. At the end of the realimentation period, there was no significant

difference in Me_mLn between the three treatment groups and these requirements did not differ significantly from those recorded at the start of the experiment.

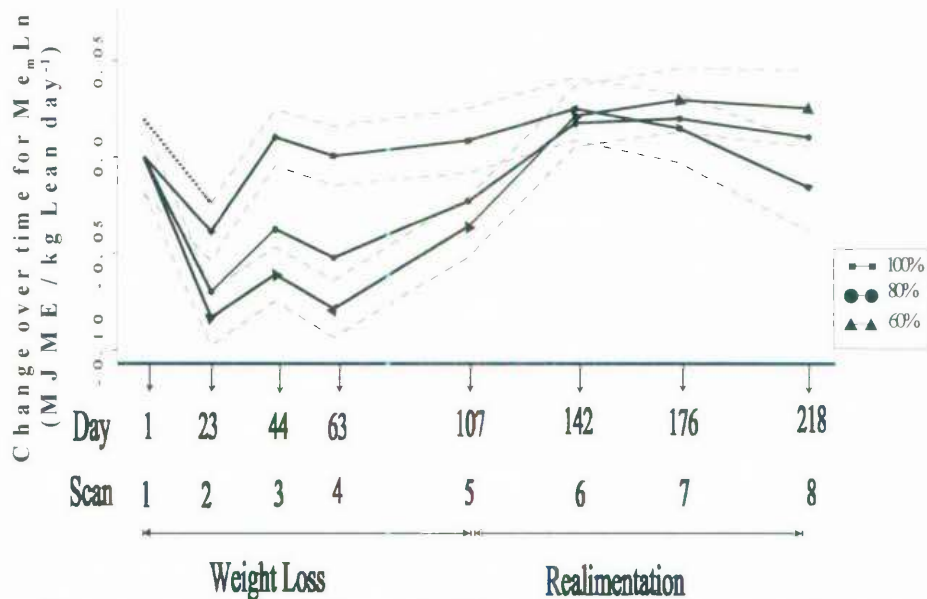


Figure 4.3.2 Deviations for Me_mLn ($MJ ME / kg LN day^{-1}$) from initial estimate for the feed required to maintain a kilogram of total body lean (scaled to zero) for the three treatment groups during the restriction and realimentation phases of the experiment. The dotted lines represents the 95% confidence limits for sheep fed at 60% and 100% of maintenance.

4.3.3 Deviations over time for Me_mEn

There were significant fixed effects of treatment*time ($P < 0.01$) and sex*time ($P < 0.05$) for the final model for the deviation over time for the feed energy required to maintain a megajoule of body energy (Me_mEn) per day. The sex and sex*time effect indicated that ewes required less energy to maintain a megajoule of body energy than rams over the entire experimental period (Table 4.3.1). A graph of the treatment*time effect (Figure 4.3.3) shows that at the end of the restriction period sheep fed at 60% of maintenance required more energy to maintain a megajoule of body energy than that required at the start of the experiment. Both random time and random animal effects were significant in the final model for the parameter Me_mEn .

The increase in the energy required to maintain a unit of body energy, that occurred during the restriction period for the sheep fed at 60% of maintenance continued through the realimentation period when fed at the original maintenance level. At the end of the realimentation period (Figure 4.3.3) these sheep required more energy per megajoule of

body energy than at the start of the experiment. This difference equated to a 37% greater energy requirement per megajoule of body energy/day than that required at the start of the experiment. Sheep that had been previously fed at 60% of maintenance during the restriction required more energy per megajoule of body energy/day than sheep that had been previously fed at 80% of maintenance during the restriction or sheep that had been fed at maintenance for the entire experimental period. Sheep fed at 80% of maintenance and at maintenance required similar levels of energy to maintain a megajoule of body energy for the entire experimental period as that required at the start of the experiment.

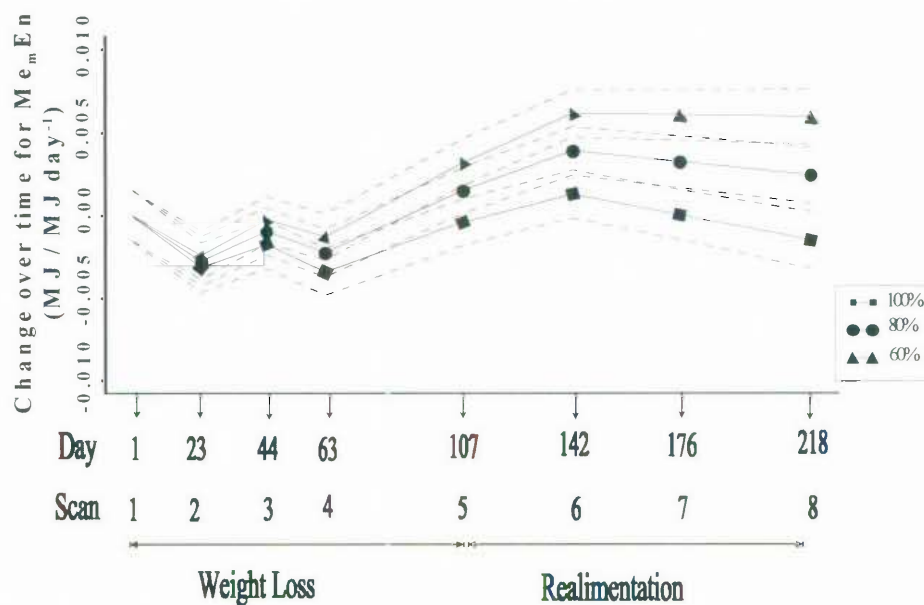


Figure 4.3.3 Deviations for Me_mEn (MJ ME/MJ day⁻¹) from initial estimate for the feed required to maintain a megajoule of body energy (scaled to zero) and the 95% confidence limits (dotted lines) for the three treatment groups during the restriction and realimentation phases of the experiment.

4.4 Discussion

Taylor *et al.* (1981) identified that Ayrshire twin cows when moved to new feeding levels after a prolonged period on a lower fixed feeding level, obtained an equilibrium weight that was proportional to the new equilibrium feeding level, raised to the exponent of one. They showed a strong correlation between concurrent measurements of this relationship between equilibrium feed intake and body weight (defined as T_0) for individual animals that did not change over a wide range of body weights from 25% to 100% of maturity. From this they indicated that the estimate for equilibrium maintenance efficiency was independent of age (except for small increases near maturity) resulting in estimates of repeatability of 0.51 for *ad libitum* feeding and 0.67 for controlled feeding.

Even though the cows of Taylor *et al.* (1981) were fed on increasing levels of feed intake, Parks (1982) extrapolated the concept of equilibrium maintenance efficiency to suggest that animals fed at below maintenance will lose weight until an equilibrium weight was reached that is proportional to the feeding level below maintenance. To test this, Parks (1982) used the data of Clappterton and Blaxter (1965) to examine the effect of a step down in feed intake from a higher equilibrium level on maintenance requirements. He showed that sheep fed at 58% of maintenance had a slightly lower maintenance efficiency ($T_0 = 9.64$ weeks) when compared to sheep fed at maintenance ($T_0 = 10.20$ weeks), although he suggested that these values did not differ significantly.

At the start of this experiment, after sheep had been fed to maintain weight for six weeks, the estimated daily metabolisable energy requirement per kilogram^{0.75} of empty body weight was 290 KJ (Ball *et al.*, 1995). This value agrees with results from Graham *et al.* (1974) for mature sheep of 300 KJ ME kg^{-0.75} day⁻¹. However these values are lower than requirements reported by Argo (1983) of between 400-530 KJ ME kg^{-0.75} day⁻¹ and Olthoff *et al.* (1989) of 614 KJ ME kg^{-0.75} day⁻¹. During the early phase of the restriction maintenance requirements did decline as shown by Gingens *et al.* (1980). Results from the present experiment agree with their value of 275 KJ ME kg^{-0.75} day⁻¹ in the early phase of the restriction.

In contrast, the results for Me_mKg for the sheep fed at 60% of maintenance indicate that at the end of the realimentation period, maintenance requirements had significantly increased. This suggests that the concept of T_0 is not applicable for sheep fed at levels below the equilibrium level (60% of maintenance) and then returned to original equilibrium feeding levels. The results for the parameter Me_mKg indicated that sheep fed at 60% of maintenance required 15% more feed to maintain empty body weight than that required at the start of the experiment. This result is consistent with those of Gingens *et al.* (1980) who calculated maintenance requirements of sheep at the end of a realimentation period of $374 \text{ KJ ME kg}^{-0.75} \text{ day}^{-1}$. Carstens (1995), also noted that maintenance requirements of steers increased by 14% during first 19 days of a realimentation phase.

However, despite the initial reduction in maintenance requirements at the start of the weight loss phase for the sheep fed at 80% of maintenance, by the end of the restriction period there was no difference in Me_mKg , when compared to the start of the experiment. Thus it appears as though the level of the severity of a restriction can cause deviations to the concept of Taylor and Turner (1968) and later Parks (1982). In this experiment prior feeding levels did effect estimates for maintenance efficiency in mature sheep that had experienced a severe feed restriction.

For sheep that were fed at 60% of maintenance, the feed required to maintain empty body weight, after adjusting for changes in body energy, declined during the initial phase of the weight loss period. During this period, there was a substantial loss of both carcass muscle and visceral lean. This may have contributed to the lower feed requirements as a greater proportion of the empty body weight during this phase was composed of adipose tissue that has a lower energy requirement to maintain when compared to the lean depots (Olthoff *et al.*, 1989). As the level of lean tissue loss approached zero and the fat mobilisation increased the relative feed requirements for empty body weight maintenance increased. This indicates that animals experiencing a weight loss phase do not always have lower maintenance requirements as previously reported (Keenan *et al.*, 1969; Gingens *et al.*, 1980; Ferrell *et al.*, 1986). In addition,

maintenance requirements per kilogram of empty body weight when adjusted for differences in body energy loss are affected by the relative proportions of tissues that are lost from the body during the weight loss phase. Recently, Ortigues and Durand (1995) showed that the maintenance requirements and total body O₂ consumption, when expressed per kilogram of metabolic weight, did not differ between ewes fed at maintenance and ewes fed at ½ maintenance. They did indicate that there were changes in the contribution of the body tissues to maintenance estimates, with the contribution of the portal drained viscera declining and the contribution of the hindquarters increasing. This adaptation of muscle metabolism to undernutrition may be a mechanism contributing to the increase in activity that was noted by Verstegen *et al.* (1982) and Olthoff *et al.* (1989) when animals were restricted in feeding level.

Many studies have shown, maintenance requirements may be more closely related to the proportion of total body lean that is present within the body (Graham, 1967; Ferrell *et al.*, 1979; Tess *et al.*, 1984). The parameter Me_mLn examined the differences in maintenance requirements after adjusting for the differences in the weight of total body lean. The decrease in Me_mLn during the early phase of weight loss mirrored the rapid loss of carcass muscle observed during that period (Chapter 3). It appears that scaling maintenance requirements per kilogram of total body lean, reflects the depletion of these tissues in the present experiment. This result is consistent with the observations of Waghorn and Wolf (1984) who stated that the fractional protein turnover rate is reduced in sheep fed a lower plane of nutrition. There was no difference in the observed maintenance requirements between the sheep fed at 80% and the sheep fed at 60% of maintenance. This result agrees Graham (1967) who stated that differences in the rate at which sheep lost weight during undernutrition could not be accounted for in terms of measured metabolic rates.

Graham (1964) stated there was no evidence to suggest that energetic efficiency increases during weight loss and there is perhaps an increased rate of protein turnover in sheep that had lost weight. In contrast Ferrell (1995), reported that reductions in maintenance requirements, in response to feed restriction, range from 10-50%. The results

for the sheep fed at 60% of maintenance support the hypothesis that maintenance requirements do vary with feed restriction when expressed either on a weight or energy basis. They do show, that maintenance requirements may not always decline with feed restriction as energetic requirements were similar at the end of the weight loss period. This result agrees with the results of Parks (1982) using the data of Clapperton and Blaxter (1965). Despite protein turnover costs being a minor contributor to muscle tissue expenditure, the increase in Me_mKg during the realimentation period is consistent with the concept that there is increased protein turnover within the carcass and visceral tissues as proposed by Graham (1964). Although Me_mLn , requirements are the similar which suggests that total body protein turnover costs are lower (Waghorn and Wolf, 1984). It is interesting to note that Bull *et al.* (1976) also observed lower maintenance requirements in ewes fed at higher levels of feed intake, whilst the reverse was reported for rams, with lower maintenance requirements being recorded at the lower feeding level. In the present experiment there was no change in Me_mLn for the sheep fed at maintenance. This contrasts with recent results of Kolstad and Vangen (1996) who showed that maintenance requirements when expressed per kilogram of total body lean declined for pigs that had been fed at maintenance for 8 weeks.

When the sheep, fed at 60% of maintenance, were returned to original feeding levels for the realimentation period, the value for the derived variable of Me_mKg increased with respect to the estimates prior to and at the end of the restriction period. This increase in the relative requirements to maintain body weight during the realimentation phase may be related to the shift to fat deposition that was noted in Chapter 3, and previously in restricted animals by Greef *et al.* (1986b) and Dulloo and Girardier (1993). Dulloo and Girardier (1993) reported that the transient shift in feed utilisation during weight loss, as shown in the present experiment, favours an acceleration of fat deposition during the realimentation period. Estimates for maintenance requirements for the sheep fed at 80% of maintenance during the realimentation period, did not differ from those estimated at the start of the experiment.

It is interesting to note that during the realimentation period, there was a small gain in fat weight and no relative change in carcass muscle weight. When maintenance requirements were expressed per kilogram of total body lean, there were no differences between the three treatment groups at the end of the experiment, despite there being clear differences in the weights of carcass muscle at the end of the experimental period (Chapter 3). This supports the concept that maintenance requirements are primarily affected by the weight of the lean tissues within the body as proposed by Graham (1967). The results obtained for Me_mLn during both the restriction and realimentation periods for those sheep that had lost weight, are consistent with the results of Gings *et al.* (1980) who showed that maintenance requirements were lower during restriction than during realimentation.

It must be noted that the calculations for Me_mKg , Me_mLn and Me_mE_n assumed that the efficiency for fat (k_f) and lean deposition (k_p) were 0.7 and 0.2 respectively and that these values were constant during weight loss and weight gain. Gings *et al.* (1980) suggested that k_f increased during realimentation due to the protein sparing mechanism that was enacted during weight loss. Ledin *et al.* (1983), Schardereit *et al.* (1995) and Kamalzadeh (1996) also reported that the efficiency of protein deposition (k_p) increases during the realimentation period in growing animals. If this did occur in the mature animals of the present experiment, then the value for Me_mKg and Me_mE_n would be lower than the value presented. However there is no evidence from the literature indicating that the efficiency of protein deposition increases during realimentation for mature animals. Further research is required to understand the effects of changing feed levels on the efficiencies for both fat and lean deposition and depletion and their associated effect on estimates of maintenance requirements.

This experiment shows that the feed required to maintain a kilogram of body weight was 16% lower for ewes than for rams. Ball *et al.* (1995) examined the feed requirements for both ewes and rams from high and control backfat lines after a period of maintenance feeding. They concluded that differences between the lines could be accounted for by differences in the relative proportions of total fat and carcass muscle

within the body. In contrast the sex effect remained at 11% after adjusting for differences in the relative proportions of body components.

For sheep fed at maintenance there were no deviations in the parameters of Me_mKg , Me_mLn and Me_mEn after 218 days of feeding. This suggests that maintenance efficiency for mature, when expressed per unit of body weight or energy, was constant. This result contrasts with the results of Graham and Searle (1975), Ledger and Sayers (1977) and Foot and Tulloh, (1977), who showed that the feed requirements for body weight maintenance decline over time. However their results were for immature animals. Turner and Taylor (1983) suggested, that a lag for the metabolic response to a decline in feeding level could account for the decline in feed requirements for maintenance over time when animals are reduced from *ad libitum* to maintenance feeding levels. It is likely that maintenance requirements are not constant for growing animals (Webster, 1977), however as animals approach maturity estimates for true maintenance requirements are more constant resulting in feed requirements remaining steady as shown by Cammell *et al.* (1993), Tolkamp and Ketelaars (1994) and Luiting *et al.* (1995).

When examining the results of the present experiment it must be remembered that the derivation of the parameters Me_mKg , Me_mLn and Me_mEn assumed that efficiency of energy utilisation for retained energy was constant. Ferrell (1995) indicated that the efficiency of energy use is maximum at maintenance but decreases non-linearly as feed intake increases above or decreases below maintenance. Thus it is possible that the change in maintenance requirements over the restriction and realimentation phases may be confounded by a non-linear change in efficiency for energy loss or gain. At present, most of the feeding standards used to calculate maintenance requirements assume there is a constant efficiency of use of feed intake or energy for gain or loss (ARC, 1980; SCA, 1990; NRC, 1996). As a result the implications of the results from the present experiment are applicable when evaluating these standards. A comparison of the results obtained for Me_mKg and Me_mLn and the differences that exist in their interpretation, clearly show that care must be exercised when maintenance requirements are scaled for different biological

parameters. Obviously, further work is required to understand the impact that changes in feeding levels has on estimates for maintenance requirements.

4.5 Conclusion

The relationship proposed by Taylor and Turner (1968) between equilibrium feed intake and equilibrium body weight may be constant for animals that are increasing in weight. For mature sheep a different equilibrium body weight and body energy content was achieved at the same equilibrium feed intake after a period of weight loss. Severe weight loss for sheep resulted in greater feed requirement for both empty body weight and total body energy maintenance, relative to requirements prior to a period of restricted feeding. Although maintenance requirements decreased during the feed restriction period for the sheep fed at 80% of maintenance, by the end of the restriction and throughout the realimentation period, the equilibrium weight that had been reached was consistent with the concept of a constant T_0 . This suggests that an increase in the severity of the feeding restriction will cause a deviation to a constant T_0 . It does appear there is a constant proportion between maintenance requirements and total body lean for mature sheep.

Estimates of feed and maintenance efficiency in mature animals must consider variations that have been imposed by prior feeding levels. From these results it is clear that nutritional history may affect the ability to accurately predict and select for maintenance efficiency in mature animals.