A STUDY OF THE METABOLISM OF LOW AND HIGH BACKFAT SOUTHDOWN SHEEP AT TWO LEVELS OF ENERGY ALLOWANCE

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PETER FRANCIS MORGAN

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TABLE OF CONTENTS

CHAPTER II: EXPERIMENTAL

EFFECT OF ENERGY BALANCE ON RESPONSES TO INTRAVENOUS HORMONE AND METABOLITE CHALLENGES IN SOUTHDOWN SHEEP FROM LINES DIVERGENTLY SELECTED ON THE BASIS OF BACKFAT DEPTH

LIST OF TABLES

LIST OF FIGURES

FIGURE PAGE

LIST OF ABBREVIATIONS

ANOVA	analysis of variance
BMR	basal metabolic rate
BSA	bovine serum albumin
BW	body weight
CV	coefficient of variation
dl	decilitre
g	gram
$G_{(0)}$	glucose space (%)
h	hour
h^2	heritability
IGF-1	insulin-like growth factor-I
IU	international unit
kg	kilogram
K_G	glucose rate constant
LPL	lipo-protein lipase
LW	liveweight
mg	milligram
mm	milimetre
mM	millimolar
ml	millilitre
ng	nanogram
NEFA	non esterified fatty acids
P	probability
r	correlation coefficient
SED	standard error of the difference
SEM	standard error of the mean
T_3	triiodothyronine
T_4	thyroxine
pg	picogram
ug	microgram
ul	microlitre

Levels of Statistical Significance

NS	Not significant P>0.10
+	0.10>P>0.05
*	0.05>P>0.01
**	0.01>P>0.001
***	0.001>P

LIST OF APPENDICIES

CHAPTER ONE

INTRODUCTION

Body Composition as a Selection Criterion in the New Zealand Sheep Industry

The New Zealand meat industry requires that producers supply lean animals for slaughter and, accordingly, payments are structured such that excessive carcase fat is heavily penalized. This requirement is a consequence of the increasing consumer preference for lean meat of high quality, a trend which reflects medical evidence that high fat diets are unhealthy, particularly given modern sedentary lifestyles. Moreover, the modern consumer prefers products which require minimal preparation and contain little waste. Selection for lean growth has therefore become an increasingly important component of sheep selection programmes. The rate of genetic gain in these programmes is to a large degree dependent upon the ability of breeders to identify animals of high genetic merit.

Genetic merit for lean meat production can be predicted from actual composition which may be measured in the live animal by various techniques. These have been reviewed by Hedrick (1983) and include measures using specific gravity, marker dilution, conductivity and x-ray techniques. Such methods are expensive and are not applicable to large scale selection of sheep. More practical measurements have therefore been sought, which would serve as predictors of genetic merit for lean growth. These predictors must fulfili certain criteria if genetic gain is to be maximised in a selection programme. They

must be highly correlated with body composition and, since the animals under test are potential replacements, they must not be harmful or terminal. Measurements should be highly repeatable and easily made. Ideally prediction of genetic merit should be possible in the young animal, preferably prior to puberty, so allowing a reduction in generation interval through hogget mating. Predictors should also be independent of non-genetic influences such as body weight or maternal effects.

The Use of Markers to Predict Genetic Merit for Body Composition

A variety of methods have been used, or might be used, to predict genetic merit for lean growth. These include assessments made on the live animal (e.g. assessment of animal size and shape), analyses of the fat tissues themselves and examination of the physiological characteristics associated with lean tissue growth. These predictors vary in the extent to which they fulfill the criteria required to achieve the selection objectives being sought.

1. Assessment of size and shape in the live animal

Body composition may be evaluated using visual assessment of conformation, by handling stock (as is routinely carried out on-farm for lamb drafting), or by using a number of measurements of body size and shape.

(a) Visual assessment of conformation

In sheep, as with many other meat-producing animals, conformation provides little indication of lean body composition (Kempster et al. 1976). This is due largely to the inability of visual assessment to distinguish between muscling and fat. Selection which concentrates on the conformational enlargement of those body areas containing the high quality cuts of meat does not succeed in producing greater muscling in these areas. The relative growth rates of groups of muscles are fixed and these muscle groups will therefore grow in unison (Berg and Butterfield 1977). Thus such selection succeeds only in increasing the fat deposition in the desired areas, as fat is the more mobile tissue (Berg and Butterfield 1977). Visual assessment of stock for composition may be successfully made by experienced judges, but this is restricted to groups of animals within a narrow weight range and with a low level of fatness (Hedrick 1983).

(b) Handling

Handling of lambs is routinely used to assess weights and carcase fatness. Experienced handlers are able to assess fatness with sufficient accuracy to permit the identification of animals ready for slaughter. However this is not sufficiently reliable for breeding or research purposes (Kempster et al. 1976).

(c) Linear measurements

There has been only a limited application of linear measurements made on the live animal to the prediction of body fatness (Purchas 1977). With selection programmes based on linear measurements, (eg. cannon bone length, width of hip points) the primary changes to occur have been in bone length, with only minor changes in the associated muscle mass (Hedrick 1983). Height and girth have provided the best linear indication of body composition (Hedrick 1983).

2. Fat characteristics

Research directly examining the adipose tissue of animals has revealed characteristics which are sufficiently highly correlated with carcase composition to be of use as predictors of body composition in the live animal. These range from tissue volume and depth measurements with various body fat depots to histological examinations. The most successful correlate found to date has been subcutaneous fat depth. Selection on the basis of this character has achieved useful changes in body composition without detrimental effects on weight, growth, reproductive performance or fleece production (McEwan *et al.* 1984).

(a) Fat depth

The accurate measurement of subcutaneous fat depth has been made possible by technological developments in the use of ultrasound. Ultrasound probes locate boundaries where changes in tissue density occur (e.g. between skin and subcutaneous fat, and between subcutaneous fat and the underlying muscle). Measurements on sheep are most commonly carried out at the "C" site, located over the longissimus muscle (eye muscle) at the twelfth rib. Fat depth over the shoulder at the "M" site has also been used to predict body composition but with less success than measurement at the "C" location (Purchas, pers. comms.).

Moderate to high correlations (0.2-0.8) between ultrasonic fat depth and carcase composition have been found (Gooden et al. 1980; Wolf et al. 1981; Parratt et al. 1987) and ultrasonic measures of back fat depth are moderately to highly repeatable (0.56-0.72; Purchas et al. 1981). Ultrasonic fat depth is also highly correlated (r=0.91) with carcase "GR", the measurement used for carcase grading (Gooden et al. 1980). The genetic gain achieved by selection based on ultrasonic fat depth will, however, be limited by the low heritability (0.2-0.3) of the fat depth measurement (Wolf et al. 1981; McEwan 1984; Simm 1986).

Fat is inherently a late-developing tissue and differences in body composition therefore become more apparent as the animals approach maturity. Maximisation of genetic improvement and long term economic return using ultrasound is best achieved by selection based on a 10-12

month weight-corrected back-fat measurement (Simm 1986; Beatson 1987). Hence selection on the basis of ultrasonic backfat depth does not appear to be a viable option if rams or ewes are to be mated as hoggets in order to reduce the generation interval.

(b) Adipocyte size and number

The histology of adipose tissue has been examined to determine whether any cellular characteristic may be indicative of genetic merit for reduced fat deposition. The total number and size of adipocyte in sheep increase up to 12 months of age with the increase in numbers, or hyperplastic growth, being most rapid in the 8-12 month period. After 12 months of age, fattening predominantly involves changes in cell size or hypertrophic growth (Hood 1977). The size of adipocytes in this later growth phase has been found to be greater in genetically fat sheep (Kadim 1989) and pigs (Hood 1983) when compared with lean animals. No difference existed between the lines in cell numbers. Selection of animals for reduced adipocyte size may reduce total body fat based on the fact that adipocyte diameter has a heritable component and a high genetic correlation with body fat content (Ch'ang et al. 1986). However, as with the use of fat depth measures, cell size would not be of use as a predictor of genetic merit for lean growth until after puberty. Problems also exist in achieving repeatable measures of apipocyte size as variation in cell characteristics exists between and within fat depots. Cell size is also influenced by nutritional status (Leat and Cox 1980).

3. Physiological characteristics

None of the currently available markers is ideal as a predictor of genetic merit for lean meat production. Superior markers are being sought which may overcome the current problems of low accuracy and high associated costs. Many attempts involve the use of divergently selected lines of animals in which the physiological basis of phenotypic differences in lean tissue growth is studied. Although the genetic merit of individual animals within these lines is not known, line means are sufficiently different phenotypically to give researchers some assurance that they also differ in mean genetic merit. It is therefore assumed that physiological differences between the lines are genetically based. Hence many attempts are being made to identify physioloigical differences between selection lines with a view to using such differences as predictors of genetic merit. Further areas of use for such selection lines are the development of exogenous growth promoting agents and the location and manipulation of key regulating genes.

Physiological Differences Between Selection Lines

1. Basal concentrations of hormones and metabolites

The measurement of basal plasma concentrations of hormones and metabolites is often the starting point in the study of physiological differences between selection lines. Such concentrations are obtained from samples normally collected by venipuncture or, for multiple or frequent sampling, by means of venous cannulae. Basal hormone concentrations may indicate the level of endocrine stimulation of a pathway which affects the growth of adipose or lean tissue. Basal metabolite concentrations may indicate the availability of intermediary metabolites for the synthesis of these tissues or level of byproducts from this synthesis. The concentrations of these factors will in turn be a function of:

a) the rate of turnover in the plasma pool,

b) the size of the plasma pool, and

c) the set level at which secretory and/or clearance processes are invoked thus modifying the concentration.

Hormones

(a) Somatotropin

Studies involving the exogenous use of somatotropin in growing animals have reported decreases in carcase fat and increases in carcase protein content in pigs (Machlin, 1972) and wether lambs (Wagner and Veenhuizen 1972; Muir et al., 1983). Increases in growth rate (Machlin, 1972; Wagner and Veenhuizen 1972) and decreased feed per

unit gain (Machlin, 1972; Muir et al., 1983) were also observed. Somatotropin treatment appears to increase carcase protein deposition at the expense of adipose tissue accretion. Further manipulation of somatotropin has been achieved by the immunisation of animals against somatostatin. This is a neuro-peptide which decreases the pituitary release of somatotropin and thyroid stimulating hormone. However, immunising lambs in this manner has not been successful in altering body composition. Some of these studies resulted in increases in growth rate (Spencer et al. 1983; Bass et al. 1984) while others obtained no response (Varner et al. 1980; Bass et al. 1984).

There is evidence of a reduction in somatotropin secretory capacity in obese humans (Bray and York, 1971) and rodents (Beck et al., 1971). Similar differences have also been noted in lines of pigs selected on backfat depth (Wangsness et al., 1977, 1981), or on both backfat depth and growth rate (Althen and Gerrits, 1976a, 1976b; Ringberg Lund-Larsen et al., 1975). Measurements of basal somatotropin levels in cattle have been negatively related to body fatness (Trenkle et al., 1978), although evidence of this relationship in ruminants is generally not well supported. However, there are difficulties in assessing the basal somatotropin status of animals as the hormone is secreted from the pituitary gland in pulses (Klindt et al. 1985). Intensive blood sampling is therefore required to monitor these pulses. The effect of somatotropin on metabolism is a function of the pulse frequency and amplitude as well as the average baseline concentration.

(b) IGF-I

The small peptide hormone insulin-like growth factor-I (IGF-I, somatomedin-C) is important in the maintenance of normal post-natal growth (Hall and Sara, 1983) and is considered to have a role in the genetic regulation of somatic growth. This is seen in the growth patterns of lines of mice selected on the basis of plasma IGF-I concentrations. Differences in plasma IGF-I levels, growth rate and mature body weight have been found in these lines (Blair et al. 1987, 1988a; Siddiqui et al. 1989a). However, Siddiqui et al. (1989b) reported that mice from low and high IGF-I selection lines had equal fat and protein composition when compared at equal body weights.

Divergence in plasma IGF-1 levels has not been found in the Massey University lines of sheep selected on weight-corrected back fat depth (Carter et al., 1989). This contrasts with greater basal levels of IGF-1 found in lean, fast-growing pig selection lines when compared with their fat, slow-growing counterparts (Ringberg Lund-Larsen et al., 1975; Althen et al., 1976). The inclusion of growth rate in the selection criteria for these pig lines may account for the divergence in IGF-1 levels. Selection on weight-corrected body composition in the Massey University sheep has not been associated with any change in growth rate (Kadim, 1989). Carter et al., (1987) found that basal IGF-1 levels were correlated with growth rate from birth to 8 months of age ($r=0.57$, $p<0.05$). This relationship has also been observed in sheep (Olsen et al., 1981) and cattle (Ringberg Lund-Larsen et al., 1977). Differences in basal IGF-1 levels in pig growth rate selection lines (Ringberg Lund-Larsen et al., 1975), and growth rate differences

in mouse IGF-1 selection lines (Blair et al., 1987) support the hypothesis of a genetic basis to the relationship between growth rate and IGF-1 levels. IGF-1 therefore appears to be associated with growth rate while having no apparent role in the determination of body composition.

(c) Insulin

In humans, elevated basal blood insulin levels have been associated with obesity (Bray and York, 1971). This association is thought to be a result of a relative tissue insensitivity to insulin and/or increased pancreatic sensitivity to glucose (Waghorn et al., 1984). Insulin is known to stimulate fatty acid synthesis and glucose utilisation in ruminants but these effects are generally less marked than those found in monogastrics (Vernon, 1980). This reduced response to insulin may be due to a low capacity for glucose metabolism in adipose tissue arising from the inability to use glucose for fatty acid synthesis and a low capacity for glucose oxidation other than via the pentose phosphate pathway.

A slight elevation in basal blood insulin concentration has been associated with increased levels of fatness in: sheep (Munro and Geenty, 1984; McCann et al. 1985c); fat milkfed vs lean early weaned lambs (Munro, 1984); fat vs lean adult sheep (McNiven, 1984); fat vs lean selection lines of pigs (Wangsness et al., 1977; Mersmann et al., 1982); and in obese vs lean heifers (McCann et al., 1985b, 1986). McCann et al., (1986) suggested that the difference in relative insulin insensitivity between the groups of heifers was a

result of decreased numbers of insulin receptors. No differences in basal insulin concentration have been found in the Massey University Southdown selection lines (Bremmers et al., (1988).

(d) Thyroid hormones

The thyroid gland maintains the level of metabolism in tissues to an optimal rate for their normal function. The thyroid hormones that achieve this, thyroxine (T_4) and triiodothyronine (T_3), stimulate oxygen consumption in most cells in the body, contribute to regulation of lipid and carbohydrate metabolism and are necessary for normal growth and maturation (Ganong, 1981).

The possibility that divergent pig selection lines differ in thyroid hormone activity and thus in metabolic rate has been examined.

Higher levels of the more active thyroid hormone T^3 have been found in the lean pig lines (Bakke and Tveit, 1977; Standal et al. 1980; Yen et al. 1985). McCann and Reimers (1986) failed to find any differences in T3 and T4 levels between lean and obese heifers, or any correlation with fatness. Obesity in humans does not seem to be associated with any abnormal levels of thyroid hormones on the blood (Glass et al., 1981).

The role of the thyroid hormones in the growing animal is considered to be permissive, and related mainly to protein synthesis (Goldberg et al., 1980; Florini, 1985). However, the thyroid hormones may affect body composition indirectly. There appears to be

some interaction between thyroxine and the effects of somatotropin and the IGF's on other tissues. It is thought that the thyroid hormones may regulate the number of muscle IGF receptors (Spencer 1985), positively interact with somatotropin releasing factor in stimulating pituitary production of somatotropin (Leung et al., 1985) and be a necessary co-factor for somatotropin-stimulated synthesis of IGF's (Froesch et al., 1985). It is not clear how these indirect actions of the thyroid hormones may affect the determination of an animal's body fat content.

Metabolites

(a) Urea and creatinine

Ram hoggets from the low backfat sheep selection line at Massey University have consistently exibited a lower basal plasma urea concentration than the high backfat selection line animals (Bremmers et al., 1988; Carter et al., 1989; Van Maanen et al., 1989). Similar differences have also been reported in pig lines selected for low vs high fatness (Mersmann et al., 1984); in Romney sheep selected for high fleeceweight vs controls (McCutcheon et al., 1987; Clark et al. 1989); in cattle selected for low vs high milk yields (Sejrsen et al., 1984; Sinnett-Smith et al., 1987); in lean Texel lambs compared with lambs from the fatter Suffolk breed (Fitzsimons and Hanrahan 1986). In each case, the low plasma urea level was evident in the high- producing selection line.

Most plasma urea is derived from liver conversion of ammonia produced by the deamination of surplus amino acids and rumen

degradation of dietary protein. It has been suggested that the observed difference in plasma urea concentrations could be due to a more efficient use of amino acids for protein synthesis in the higher producing lines, with an associated reduction in the rate of deamination of amino acids. However, when studying Romney fleeceweight and control lines, McCutcheon et al. (1987) and Clark et al. (1989) observed that the fleeceweight line also maintained a lower plasma creatinine concentration. This suggests that the control line sheep had a lower glomerular filtration rate (GFR) since this is often a characteristic of animals with high levels of substances such as urea and creatinine which are not actively secreted or reabsorbed in the kidney nephron. Experiments carried out by Bremmers et al., (1988) in the high and low backfat Southdown selection lines and by McCutcheon et al., (1987) in the Romney fleeceweight and control lines, have failed to show any differences between lines in urea excretion rate or creatinine clearance rate. The plasma urea differences were, however, no longer significant when corrected to a common creatinine clearance rate and urea excretion rate. In a more thorough study of urea metabolism in the Southdown selection lines, Van Maanen et al. (1989) found that the plasma urea differences between the lines appeared to be primarily due to differences in urea clearance rate from the kidney. However, in contrast to the fleeceweight selection lines (McCutcheon et al. 1987; Clark et al. 1989) there were no differences in creatinine clearance rate or plasma creatinine concentration between the Southdown lines. Thus the differences in urea clearance cannot be explained entirely by differences in glomerular filtration rate.

(b) Glucose

Bremmers et al. (1988) found an elevated basal blood glucose concentration in the low backfat selection Southdown line of sheep compared with high backfat sheep. This difference existed in spite of a lower energy intake in the low backfat animals due to diet selection. Such differences have not been found in other studies involving the same selection lines (Carter et al. 1989; Van Maanen et al. 1989). Differences have been found in pig selection lines where obese pigs maintained higher levels of blood glucose, but these levels were not sufficiently different to be considered a reflection of abnormality in glucose metabolism (Steele et al., 1972). Subsequent studies have failed to find differences in basal blood glucose concentration in pig selection lines (Cote et al., 1982).

(c) Non-esterified fatty acids

McNiven (1984) found that in a group of fat sheep (after 3 months on a high plane of nutrition) the non-esterified fatty acids (NEFA) levels were higher than in leaner sheep. However this study reflected phenotypic, rather than genotypic, differences in body fatness. No differences in basal blood NEFA concentration have been found in relation to fatness in sheep selection line studies (Bremmers et al., 1988; Carter et al., 1989). Similarly, blood cholesterol and triglycerides (at normal feeding levels) have not been found to vary between pigs selected for rate of gain and backfat thickness (Bakke, 1975).

Measurements of baseline concentrations of hormones and metabolites may not be ideal as physiological markers of body composition. They are affected by external enviromental factors, are sometimes difficult to interpret, and are constantly being modified by homeostatic regulators. Moreover, the concentration of a particular hormone or metabolite in the plasma pool may not reflect the dynamics of the system because of homeostatic intervention. Many situations are known in which marked changes in metabolic function occur in the absence of changes in hormone or metabolite concentrations. These long-term changes in metabolism have been termed "homeorhesis" (Bauman and Currie, 1980). Techniques other than simple measurements of metabolite/hormone concentrations in plasma are required to investigate these changes.

Responses to Metabolite and Hormone Challenges

It has been proposed that animals achieve the dynamic control of nutrient partitioning by the altering the sensitivity of specific target tissues to homeostatic regulators. (Bauman and Currie, 1980) Challenges are therefore used to detect differences between genetic groups in sensitivity to homeostatic signals. Challenges generally involve the measurement of basal levels of hormones and metabolites prior to the administration of the challenge and the subsequent monitoring of responses in a further series of samples until basal concentrations are again reached or approached

(a) Insulin challenge

The higher basal glucose concentration found in the low backfat sheep selection line by Bremmers et al. (1988) despite the similar basal insulin concentration in the two lines may reflect a reduced peripheral sensitivity to insulin. If this were the case, the blood glucose response to an insulin challenge would be expected to be less in the low backfat line. When animals from the same lines were challenged with insulin by Carter et al. (1989) no such differences were evident. Conversely McCann and Reimers (1986) found that obese heifers had a reduced glucose response to insulin and a greater basal insulin concentration, suggesting that they are resistant to insulin.

(b) Glucose challenge

The lower basal insulin to glucose ratio found by Bremmers et al. (1988) in low backfat sheep could also be interpreted as suggesting that the pancreas of the low backfat line sheep is less responsive to circulating glucose than that of the high backfat line. McCann et al. (1985c) found that obese Dorset ewes had a greater glucose and insulin response to a glucose challenge when compared with lean ewes. A greater insulin response to glucose has been also been found in obese compared with lean heifers (McCann and Reimers, 1985). Carter et al. (1989) were unable to detect differences in pancreatic sensitivity (ie.insulin response) to a glucose challenge in their work with the Southdown selection lines. However the high backfat line had a greater plasma glucose response to the glucose challenge which appeared to have been largely attributable to their smaller glucose distribution space. McNiven (1984) found a smaller glucose distribution space and pool size in a group of phenotypically lean

crossbred sheep compared with fat sheep.

Work by Francis et al. (1988) and Armstrong et al. (1988) has produced lines of lambs sired by rams selected for either long or short half life ($T_{1/2}$) of glucose in plasma following an intravenous glucose infusion. The progeny of the rams with the longer $T_{1/2}$ have a lower subcutaneous fat level and a greater rate of growth. The longer $T_{1/2}$ group also have a reduced hypoglycaemic response to exogenous insulin.

(c) Glucagon challenge

Glucagon is a hormone secreted primarily by the pancreatic A cells although it is also synthesized by duodenal and stomach tissue (Ganong 1981). Glucagon is a glycogenolytic, gluconeogenic and lipolytic hormone. Its main actions are therefore to increase blood FFA levels and, by activating liver adenylate cyclase, which stimulates liver phosphorylase activity, it stimulates glycogen breakdown to glucose. The presence of glucagon stimulates the production of somatotropin, insulin and somatostatin (Ganong 1981) while glucagon secretion is inhibited by glucose and amino acids. Since insulin is a glycogenic, antilipolytic, and antigluconeogenic hormone, these energy-storing actions oppose the energy-releasing actions of glucagon. The insulin-glucagon molar ratio, normally about 2.3, therefore determines the net effect that these hormones have on carbohydrate and lipid metabolism.

Carter et al. (1989) found a greater plasma NEFA response to a

glucagon challenge in the low backfat Southdown sheep selection line compared with the high backfat line. This suggests that the low backfat line is more sensitive to the lipolytic effects of glucagon than the high backfat animals. No differences in blood glucose response to glucagon were apparent between the lines.

(d) Adrenaline challenge

Adrenaline (epinephrine) is secreted from the adrenal medulla and among its actions on metabolism are lipolytic and glucogenolytic effects.

Carter et al. (1989) failed to find differences between the Southdown selection lines in lipolytic response to an adrenaline challenge (ie. the adrenaline response did not differentiate between the lines in the same way that lipolytic responses to glucagon did). However pig studies have found that fat slow-growing pigs are less sensitive to the lipolytic effects of adrenaline than lean fast-growing lines (Standal et al. 1973, 1979; Mersman 1985). Brocklen et al. (1986) also showed a reduced lipolytic response to an adrenaline challenge in fat Pietrain pigs when compared with lean pigs. Cellular response to glucagon and adrenaline is mediated via the C-AMP cascade system involving specific receptors for these hormones (Hu et al. 1987). Differences found by Carter et al. (1989) in lipolytic response to glucagon, but not to adrenaline, may reflect a greater number or binding affinity of glucagon receptors than adrenaline receptors in the low backfat line.

(e) Fasting and refeeding

The deprivation of an external nutrient supply will induce many physiological changes which enable the continued supply of necessary metabolic substrates. These changes involve the mobilisation of stored nutrients, particularly stored energy, and a reduction in the accretion of many tissues. The physiological response to fasting requires a coordinated change in the partitioning of available nutrients which may differ between differing genotypes for body composition. In a study of the Massey University Southdown selection lines (Carter et al. 1989) animals were fasted and refed and physiological responses were monitored. The somatotropin response to fasting of the low backfat animals was three times that of the high backfat animals. Similar fasting response differences have been found in lean and obese lines of pigs (Wangsness et al. 1977, 1981). Fasting and refeeding urea differences between the sheep selection lines have also been found with lower urea levels in the low backfat animals (Carter et al. 1989). No differences have been found in the changes in glucose and NEFA levels associated with fasting in the sheep (Carter et al. 1989) or pig lines (Wangsness et al. 1977, 1981).

4. Fat breakdown and oxidation

The rate of adipose tissue development is a function of both lipogenesis and lipolysis. There are a number of key enzymes controlling these opposing processes which have the ability to exert an influence over the determination of body composition. The activity of some of these enzymes may display sufficient genetic variation to enable their successful use as genetic markers in selection programmes for meat-producing animals.

Many studies of the rate of adipose tissue breakdown (lipolysis) involve the measure of rate of epinephrine-stimulated fatty acid release. This primarily measures the activity of the fat mobilising lipase enzyme, responsible for the hydrolysis of fatty acids and glycerol from triglycerides. Many pig studies have examined lipolytic rate in selection lines (Scott et al. 1981; Mersmann et al. 1984; Mersmann and Koong 1984) and have found no consistent differences between lines. Mersmann (1985), in an effort to resolve the assessment of lipolytic rate in the pig lines, conducted a large trial on lean and obese pig lines and was not able to detect any differences.

Catalase, an enzyme involved in many pathways including fatty acid oxidation in the liver, has been found to have a greater activity in liver biopsy samples from high backfat Southdown sheep of the Massey University selection lines, compared with the low backfat sheep (Peterson and Purchas, 1989). The correlations between liver catalase activities in 10 sires (5 sires per line) and both the ultrasound "C"

fat depth and carcase "GR" fat depth measurement of 293 progeny were 0.60 ($P=0.05$) and 0.65 ($P<0.05$), respectively. Due to the many metabolic processes involving the catalase enzyme further work is necessary to verify the findings of Peterson and Purchas (1989).

5. Fat accretion

Lipoprotein lipase (LPL) is an enzyme which catalyzes the uptake of fatty acids at the blood capillary interface. A greater foetal activity of LPL has been found at 110 days of gestation in high backfat lines of pigs compared with the low backfat line animals (McNamara and Martin, 1982). The larger average adipocyte size found by Hausman et al. (1983) in the high backfat pigs of the same lines at the same age would also support this difference in lipogenesis. Measures of postnatal lipogenesis in pigs have also identified greater rates in pigs from lean selection lines at 3-6 months of age (Steele et al., 1974; Steele and Frobish, 1976; Scott et al., 1981).

The supply of reducing equivalents (NADPH) has been found to be a major factor limiting the de novo synthesis of fatty acids. The pentose phosphate pathway produces 50-80% of NADPH required in pig adipose tissue for the synthesis of fatty acids. This pathway is catalyzed by the enzymes glucose-6-phosphate dehydrogenase (G-6-PD) and 6 phosphogluconate dehydrogenase (6-PGD). The remaining NADH is produced by the oxidation of citrate to pyruvate in a pathway catalysed by malic dehydrogenase (MD), citric cleavage enzyme (CC) and NADP isocitrate dehydrogenase (NADP-ICD). Elevated activities of these enzymes has been reported in lines of pigs selected for fatness

(Steele et al., 1972; McNamara et al., 1982; Rogdakis, 1982; Rothfuss et al., 1984) and in obese rats (Hood, 1983). The activity of these enzymes has been shown to be highly heritable in pigs (h^2=0.4-0.7, half sib analysis) and to have a genetic correlation with carcase fatness of 0.4-0.7 (Strutz et al., 1972; Standal et al., 1979).

6. Protein synthesis

Final muscle mass is determined early in life by the replication and accumulation of DNA by muscle satellite cells. The amount of DNA replicated may be related to the number of precursor satellite cells at or near birth (Allen et al. 1979) Lower numbers of satellite cells showing proliferative activity have been found in high fat lines of pigs (Hoffman et al. 1983) and mice (Purchas et al. 1985). The rate of muscle thymidine uptake has also been related to rate of DNA synthesis. Lord et al. (1986) found an increased rate of incorporation of labelled thymidine into the muscle of young lambs from a low fat selection line. Both these findings suggest that the replication of DNA and therefore the determination of potential muscle content may vary between genotypes for lean body content.

Post-natal muscle hypertrophy is the net result of muscle accretion and degradation, the ratio of which, in young pigs, is approximately 2 to 1 (Waterlow et al. 1984). Insulin is necessary for the control of amino acid uptake in muscle and the supply of energy, and is a co-factor to somatotropin for the synthesis of IGF-I in the liver (Allen et al. 1979). However insulin has more often been negatively

associated with protein content and positively associated with fat content (Trenkle et al. 1978) and is therefore considered as having permissive role in protein accretion.

Post-natal treatment of young sheep with somatotropin promotes increases in growth rate and the relative rate of protein deposition (Chung et al. 1985; Pell et al. 1987) and foetal body composition has been related to plasma somatotropin levels in genetically lean and obese pigs (Hoffman et al. 1983). Somatotropin has also been implicated in the promotion of myogenic cell proliferation in young pigs (Kotts et al. 1987). These findings are supported by consistently greater plasma somatotropin levels found in lean pig selection lines when compared with fat selection lines (Ringberg Lund-Larsen et al. 1975; Althen et al. 1976).

Muscle cell substrate uptake, ribosome activity, DNA synthesis and protein synthesis in vitro have all been shown to increase when animals are treated with IGF-I, IGF-II and somatotropin (Florini 1985; Froesch et al. 1985). Somatotropin appears to have a positive effect on protein deposition as is seen in pig selection lines (Ringberg Lund-Larsen et al. 1975; Althen et al. 1976) and it is likely that the effects of somatotropin are invoked both directly on muscle cells (Florini 1985) and via IGF-I (Ringberg Lund-Larsen et al. 1975).

Positive interactions between the thyroid hormones and the effects of somatotropin and IGF-I on protein accretion have been found, possibly due to thyroid regulation of IGF receptor numbers (Spencer 1985), positive interaction with growth hormone-releasing factor

(Leung et al. 1985), and the fact that thyroid function may be a necessary factor for hormone stimulated synthesis of IGFs (Froesch et al. 1985). Thyroid hormones have not been directly related to control of protein metabolism and therefore, as with insulin, appear to have a permissive role.

Non-Genetic Factors Affecting Physiological Differences Between Selection Lines

When attempting to identify genetic effects on physiological processes in animals, consideration must be given to interactions between genetic and non-genetic effects. The non-genetic effects, including enviromental, age, weight, etc. effects, will dictate the conditions under which studies must be performed if the isolation of true genetic effects is to be attempted. Failure to correct for or standardise non-genetic factors may mean that potential genetic markers are not identified or that selection on the basis of markers is associated with low rates of genetic gain due to the influence of the non-genetic factors (ie. a low heritability). Some examples of important non-genetic effects will now be considered.

1. Age

Van Maanen et al. (1989) found a relationship between plasma urea levels and age in Southdown sheep from the Massey University selection lines. While urea in the low backfat line remained independent of age (over the range of 6 to 8 months), levels in the high line decreased with age. Thus urea levels in the two lines converged as the animals

aged. This relationship was not been found in two previous studies on the same sheep selection lines (Bremmers et al. 1988; Carter et al. 1989), possibly because fewer animals were involved and the range in ages was smaller. Sejrsen et al. (1984), in a study of bull calves of high or low genetic merit for milkfat production, observed large between-line differences in plasma urea concentration at about 3.5 months but only small differences at 7 months of age.

When studying changes in the glucose-insulin status of pig selection lines, Mersmann et al. (1982) showed that found that plasma insulin in the fat and lean lines diverged with increasing age. Pigs from the lean line had lower insulin levels than those of the fat line at 16-22 weeks of age but not at 4-12 weeks. Wangsness et al. (1977) found a greater increase in glucose clearance rate with age in lean selection line pigs than in obese pigs.

Differences in the rate of lipolysis between selection lines of pigs may also be dependent on age (Mersmann 1986). When lipolytic rates were compared for pigs at 28, 42 and 56 days, the obese pigs had greater rates of lipolysis only until 42 days old. At 56 days, lipolytic rates were similar in the two lines.

Thus age is a factor which can affect the magnitude of genetically based differences in metabolism related to body composition. Evidence of this genotype by age interaction implies that care must be taken when selecting the age at which animals are to be studied. The effects of the genotype by age interactions appear to be most apparent when an animal is young and growing rapidly. However, in the growing

animal, the interactions between genotype and age may be confounded with weight effects thus making the identification of any true age effect more difficult.

2. Physiological state

The main changes in the physiological states of farm animals are associated with females and include stage of the oestrus cycle, pregnancy, and lactation. Effects of puberty, another time of important physiological changes, are to a large degree confounded with the effects of age as discussed previously.

McCann and Reimers (1986) examined the relationship between the stage of oestrus cycle and insulin/glucose metabolism in phenotypically lean and obese heifers. They found that basal concentration of insulin was positively correlated with degree of obesity at both oestrus and dioestrus. However, the difference between obese and lean heifers in basal insulin was greater at dioestrus than at oestrus. There have been no attempts to examine the effects of other physiological states (eg. pregnancy, lactation) on the metabolism of genetically lean and fat animals.

3. Diurnal variation and daylength

During a 24 hour blood sampling study by Bremmers et al. (1988) with high and low backfat sheep lines, plasma NEFA displayed a diurnal pattern in rams fed the 0.7 maintenance ration even though the animals were fed at 2 hourly intervals and remained under a constant lighting

regimen. Thus the low backfat animals had a greater basal NEFA concentration but only during the night. There is also evidence of a photoperiodic/seasonal effect on blood urea differences between Romney fleeceweight selection lines (Matthew 1989). The difference in blood urea between the fleeceweight and control lines tended to be greatest during the winter period.

4. Energy balance

The plane of nutrition is an important factor influencing most metabolic processes through the supply of nutrients. Attempts to minimize variation in energy balance require the standardization of feed intake. This commonly involves feeding all animals a fixed proportion of maintenance, assuming that maintenance can be calculated using metabolic body weight (liveweight$^{0.75}$ is often used). However, in most studies involving selection lines, plane of nutrition is "standardised" by assuming that the two lines have equal maintenance energy requirements per unit metabolic liveweight. This assumption does not always hold true. For example, lean and obese pigs fed in proportion to metabolic liveweight have been shown to have different energy balance, implying different maintenance requirements (Yen et al. 1983). Failure to standardise energy balance in metabolic studies may lead to erroneous conclusions. An example of this was seen in a study comparing basal concentrations of metabolites and hormones in high and low producing dairy cows when fed at similar energy intakes (Hart et al. 1978). Differences found between the high and low producing cows in the levels of these blood componants were largely due to differences in energy balance (ie. energy balance and genetic

merit were confounded) and differences disappeared when animals were fed to maintain equal energy status (Hart 1983).

Nutritional status could potentially be used to optimise the expression of physiological differences between differing genotypes as it not only directly affects the circulating levels of metabolites and hormones but can alter tissue sensitivity to hormones. An example of this was seen when the release of somatotropin, and its relationship with plasma IGF levels, was studied in steers fed on three different planes of nutrition (Breier et al. 1986). Associated with the lower levels of intake were a higher mean concentration, amplitude and response area, and the elimination of any diurnal rhythm in plasma somatotropin. Baseline concentration and pulse frequency did not alter with plane of nutrition. These changes are consistent with the hypothesis that nutrition-dependent change in somatotropin responsiveness is a dominant influence on the state of the somatotrophic axis and growth in the ruminant. Thus manipulation of energy balance could potentially be used to maximise differences between genotypes in the function of the somatotropic axis.

The effect of the interaction between energy balance and genotype for body composition on basal hormone and metabolite concentrations was studied by Bremmers et al. (1988). They used feeding levels of 0.7 and 1.2 times maintenance energy requirement to create differing energy balances between high and low backfat Southdown selection line sheep. They found that energy balance did influence physiological differences between the selection lines. Basal plasma urea differences between the lines were greater when animals were fed above

maintenance requirement and basal creatinine differences were greater when animals were fed below maintenance. Interactions with energy balance have not been used as criteria in metabolic studies examining responses to metabolic challenges in divergent selection lines.

In conclusion, it seems likely that a number of non-genetic factors may influence the expression of metabolic differences between animals of low or high genetic merit for lean tissue growth. Energy balance is likely to be one important such influence and may also be responsible for differences observes in different physiological states. However, despite its potential importance, the effect of energy balance on the metabolism of genetically lean and fat animals has not been studied in detail.

Purpose and Scope of the Investigation

Previous studies have shown that differences exist between high and low backfat selection lines in basal hormone and metabolite concentrations and in the physiological responses to metabolic challenges. Metabolic challenges involve the intravenous administration of homeostatic regulators such as hormones or metabolites, as well as fasting and refeeding treatments. If such differences in hormone and metabolite concentrations were both repeatable and easily measured, they could prove to be useful markers of genetic merit for lean meat growth in young animals.

The responsiveness of metabolic systems to homeostatic regulators is known to be influenced by the energy balance. Thus it is likely that the differences between the genotypes in responsiveness to challenges may vary according to the energy status. This would represents a genotype by energy status interaction. If this were so, the energy status would then represent an important consideration in the use of specific genetic markers for the differentiation of selection lines. Some examples of genotype by energy status interactions have been described previously.

The objective of this study was therefore to examine responses to metabolic challenges in ram hoggets from the Massey University high and low backfat Southdown selection lines. Of particular interest were the effect of energy balance on responses to metabolic challenges, and the interaction between selection line and energy balance. Feeding levels of 0.7 and 1.3 times calculated maintenance energy requirements were used to create divergent energy balances for the purposes of comparing the selection lines. The metabolic challenges chosen for this study were adrenaline, glucagon, insulin, glucose and a 72 hour fasting period.

CHAPTER II

EXPERIMENTAL

EFFECT OF ENERGY BALANCE ON RESPONSES TO INTRAVENOUS HORMONE AND METABOLITE CHALLENGES IN SOUTHDOWN SHEEP FROM LINES DIVERGENTLY SELECTED ON THE BASIS OF BACKFAT DEPTH

P. F. MORGAN

Department of Animal Science, Massey University,
Palmerston North, New Zealand.

Abstract

The aim of this study was to examine the effect of energy balance on responses to metabolic challenges in selection lines of sheep differing in propensity for lean growth. Twenty-four Southdown ram hoggets (12 each from the Massey University high and low backfat selection lines) were offered lucerne chaff at 0.7 or 1.2 times maintenance requirement in a balanced factorial design. The metabolic challenges involved the administration of acute intravenous doses of adrenaline, insulin, glucose and glucagon, and a 72 hour fast. Plasma concentrations of hormones and metabolites in response to these challenges were monitored by serial blood sampling.

Relative to the 1.2 times maintenance feeding level, the animals fed 0.7 times maintenance had; greater basal NEFA levels; a greater

lipolytic response to the glucagon and adrenaline challenges; lower basal concentrations of glucose and insulin; a lower glucose clearance rate in response to the glucose and insulin challenges; and higher plasma creatinine but lower urea levels during the fast.

The low backfat selection line rams had a greater reduction in plasma glucose concentration in response to the insulin challenge and a greater level of somatotropin during the fasting period compared with the high backfat animals. Higher basal levels of T_3 concentrations were also found in the low backfat rams. It is concluded that metabolic differences between the two lines are not markedly influenced by energy balance.

Introduction

Genetic merit for lean meat production may be predicted from estimates of body composition (Hedrick 1983). Selection of animals on the basis of weight-corrected backfat measurements has been used to achieve genetic improvement in lean growth (Beatson 1987). However, superior markers of genetic merit for lean growth are being sought which may overcome the problems of low accuracy and/or high associated costs with currently available markers.

Physiological differences between lines of animals selected on the basis of body composition have been examined in an attempt to identify metabolic characteristics associated with lean body growth. Such physiological markers could potentially reduce errors of prediction, so allowing earlier selection of breeding stock and increasing the rate of genetic gain.

Several physiological differences between selection lines of animals have been found. Higher levels of somatotropin have been found in lines of pigs selected for lean growth when compared with the fat selection lines (Althen et al. 1976; Wangsness et al. 1977). Plasma urea concentration has also been observed to differ between selection lines. Lower levels of urea have been found in lean vs fat lines of pigs (Mermann et al. 1984) and low vs high backfat sheep (Carter et al. 1989; Van Maanen et al. 1989). The association between lower urea levels and increased productivity has also been found in dairy cows selected for high vs low milk yield (Sejrsen et al. 1984; Sinnet-Smith et al. 1987) and in Romney sheep seleceted for high vs low fleece weight (McCutcheon et al. 1987; Clark et al. 1989).

Studies in humans (Bergman et al. 1981), pigs (Wangsness et al. 1977), cattle (McCann et al. 1986) and sheep (McNiven 1984; Munro et al. 1984) have demonstrated that obese individuals or lines have higher basal concentrations of glucose and/or insulin, reduced peripheral sensitivity to insulin and, in some cases, increased pancreatic sensitivity to glucose.

Exogenous adrenaline has been shown to provoke a greater lipolytic response in lean selection lines of pigs than in contemporary fat lines (Standal et al. 1973, 1979; Mersmann et al. 1985; Brocklen et al. 1986). An increase in lipolytic response to exogenous glucagon, but not to exogenous adrenaline, has been found in a low backfat line of sheep compared with high backfat animals (Carter et al. 1989).

Such differences between selection lines indicate that basal concentrations of hormones and metabolites may be genetically correlated with composition of growth. Differences in tissue responsiveness to homeostatic factors may also indicate differences in the dynamics of nutrient partitioning to either lean or fat tissue (Bauman and Currie 1980). Carter et al. (1989) used metabolic challenges of intravenous hormones and metabolites, and fasting, to compare response differences in sheep from two selection lines. They found differences in responsiveness to glucagon and glucose challenges which may represent potential predictors of genetic merit.

Many of the responses which have been measured in studies of lean and fat selection lines are known or expected to be strongly influenced by energy balance. Thus the possibility exists that differences between the lines depends on the energy status of animals being studied. The objective of this study was therefore to examine the effects of energy balance on differences in metabolic characteristics between the Massey University high and low backfat selection lines of Southdown sheep.

Materials and Methods

1. Animals

Twenty-four Southdown ram hoggets, 12 each from the Massey University High (H) and Low (L) Backfat selection lines, were used in this study. The lines originated from a selection experiment which started in 1976 and involved approximately 50 commercial Southdown

ewes per line. These ewes were allocated to lines, and replacements have subsequently been selected, on the basis of weight-corrected backfat depth (Purchas et al. 1981; Purchas et al. 1982) measured using an ultrasound backfat probe as described by Gooden et al. (1982). The selection lines now comprise about 80 mixed-age ewes per line and each year 18 month-old rams (2 per line) are selected for mating.

The rams in this study were chosen from those 1985-born animals which, prior to the start of the study, were greater than 25 kg liveweight and had at least 1 mm of backfat (as measured by the ultrasonic probe). They were approximately 6 months of age at the commencement of the study and equally represented the two selection lines as well as the two sires within each line.

The animals were housed in metabolism crates and were secured with chained halters. They were fed chaffed lucerne hay at rates equivalent to either 0.7 or 1.2 times their maintenance energy requirement in a factorial arrangement with selection line (see Appendix I). Animals were fed once daily at 1700 h and fresh water was available ad libitum. The individual feed allowances were calculated from experimentally-derived requirements for lambs (Rattray, 1986). Details of the calculations are in Appendix II. The daily maintenance feed requirement for a ram lamb was assumed to be 0.7 MJME/kg liveweight$^{0.75}$ (Rattray, 1986). The lucerne hay had a dry matter (DM) content of 88.4% and was assumed to have a metabolisable energy content of 9.5 MJ/kg DM (Holmes and Wilson, 1984). Crude protein content of the feed was 21.7% on a DM basis. The feed

allowance for each animal was weighed out daily on electronic scales. On alternate days, 2 g of a mineral supplement (59% sodium chloride, 37% sodium sulphate and 4% sodium molybdate) was added to the lucerne hay to counteract possible copper toxicity.

2. Experimental Procedure

To assist in the running of the trial the animals were divided into two blocks of 12 animals balanced for line and sire (see Appendix I). These blocks were run 10 days apart.

(a) Protocol

The following protocol outlines the sequence of events for each block of animals for the duration of the experiment.

Table 2.1 Protocol of events

DAY	PROTOCOL
1	Rams into metabolism crates and fasted weight taken
1-3	Fed 1700h (maintenance level)
4-10	Fed 1700h (0.7 or 1.2 maintenance)
11-21	Balance period
22-23	Fed 4 hourly and cannulated
24-25	24h blood sampling and fed 2 hourly 1700h-1700h
26-28	Fed once daily at 1700h
29	Adrenaline challenge
30	Insulin challenge
31	Glucagon challenge
32	Glucose challenge
33-35	Fasted from 0800h on day 33
36	Refeeding and removal of cannulae
38	Weighed and backfat depth measured
38	Returned to pasture

Upon entering the indoor enclosure the rams were fasted for 24 hours after which they were weighed and allocated a daily maintenance ration. After day three they were then put onto either the 0.7 or 1.2 times maintenance ration and allowed 7 days in which to adjust.

As part of another study (Bremmers et al. 1988) (Appendix III), the animals underwent an energy and nitrogen balance period and then a diurnal study involving 2 hourly blood samples over 24 hours. This was followed by the challenge and fasting periods of the current study.

The balance period entailed a 10-day collection of urine and faeces, from day 11 to day 21, to study the energy and nitrogen status of the animals. The rams were then given three days (22 to 24) to adjust to 4 hourly feeding in preparation for the two hourly feeding necessary during the 24 hours of blood sampling beginning on day 25. During these three days of adjustment, jugular cannulae were inserted. During days 25 to 26, the animals were fed after each 2 hourly blood sample, their daily ration being divided into 12 equal portions. This was then followed by an adjustment back to once daily feeding from days 26 to 28.

The four hormone and metabolite challenges were administered over the next four days (29 to 32) and the accompanying blood samples were taken. The rams were then fasted for 72 hours, from day 33 until day 35, with two blood samples being taken each day. Following refeeding and the removal of the cannulae on day 36, the rams were kept inside for observation for a further 2 days during which time a 24 hour fasted weight was taken, backfat depth measured and animals were returned to pasture on day 38.

(b) Cannulation

Jugular cannulae were inserted to facilitate sampling blood and infusion of challenges. To introduce the cannulae the rams were restrained in a small head bail and tied so as to immobilise and extend the head, exposing one side of the neck. The area over the jugular vein was clipped, washed with a dilute solution of disinfectant (Savlon, ICI Tasman, Wellington, NZ) and swabbed with 70% ethanol. A local anaesthetic (Xylocaine spray, 10% lignocaine, ASTRA Pharmaceuticals PTY Ltd., N. Ryde, NSW, Australia) was applied to the area and allowed several minutes to act. The vein was then occluded and a 14G Argyle Medicut "T" Sleeve (Sherwood Medical, St Louis, Mo., United States) passed down through the skin into the vein until the blood flowed freely. The needle was removed leaving the sleeve in the vein, and a cannula (medical grade polyethylene tube; i.d. 1.0mm, o.d. 1.5mm; Dural Plastics and Engineering, N.S.W., Australia) passed through the sleeve and 6cm into the vein. A small quantity of blood was drawn from the cannula to ensure that it lay inside the vein. The sleeve was withdrawn and a short length of tape (Sleek plastic adhesive strapping no.7045, Smith and Nephew, Auckland, NZ) was wrapped around the base of the cannula. A single linen suture was placed in the skin, 1 cm above the point of entry of the cannula, using a 16G needle (Nipro, Japan). This was tied off around the cannula, biting into the tape to prevent slipping. Antibiotic powder (Aureomycin 2%, Cyanamid, Auckland, NZ) was applied to the site and the cannula bandaged (Elastoplast elastic adhesive bandage no. 1004, Smith and Nephew, Auckland, NZ) to the neck so as to emerge behind the head. An elastic mesh (Systenet, size C, International Surgical

Netting S.P.A., Moncalieri, Italy) was then used to cover the dressing. Intramuscular antibiotic (5ml/day, Streptopen lot, 312620, Glaxo, NZ) was administered and rectal temperatures monitored daily. No elevated rectal temperatures were observed during the experiment. Patency of the cannulae was maintained by ensuring that they were regularly flushed with heparinised saline (0.9% NaCl, Travenol Laboratories NZ Ltd., Auckland, NZ; 100IU/ml sodium heparin 161IU/mg, batch 128B, Wendal Pharmaceuticals Ltd, London; 0.04ml/100ml oxytetracycline, Terramycin LA, Pfizer Laboratories, Auckland, NZ).

(c) Challenge period

Four intravenous challenges (adrenaline, insulin, glucagon and glucose) were administered over 4 consecutive days. The dose rates and concentrations of the challenge solutions are shown in Table 2.2.

Table 2.2. Dose rates and concentrations of the four intravenous challenges.

Challenge	Dose Rate (/kg liveweight)	Concentration (/ml)
Adrenaline[a]	1.0ug	7.0ug
Insulin[b]	0.01g	0.07g
Glucagon[c]	0.175ug	1.5ug
Glucose[d]	0.17g	0.4g

[a] McGaw Ethicals Ltd, Auckland, NZ.

[b] Lot 121c-1350, Cat. No. I5500, 26.4 IU/mg, Sigma Chemical Co., St. Louis, Mo., USA.

[c] Lot 735094, Sigma Chemical Co., St. Louis, Mo., USA.

[d] Dextrose 40%, NDA Animal Remedies, Auckland, NZ.

The adrenaline challenge was prepared on the morning of use. Stock adrenaline (1ug/ml) was diluted to the appropriate concentration with saline in a dark bottle and transferred to individual syringes covered with foil to exclude light.

The insulin and glucagon challenges were both prepared as follows. The hormone was weighed out (Cahn Model 4600 Electrobalance,

Cahn/Ventron Corp., Paramount, Ca., USA.) and dissolved in BSA solution (bovine serum albumin, 0.015g/ml saline, Sigma Chemical Co., St. Louis, Mo., United States). Solutions were then drawn into syringes and frozen with an airspace until being thawed on the morning of the challenge.

The glucose challenge was prepared by drawing the glucose, undiluted, into syringes on the day required.

To assist in the management of blood sampling, the 2 blocks of 12 rams were further divided into 3 groups of 4 rams (balanced for line and sire, see Appendix I). The and administration of challenges to these 3 groups was as is shown in the following table.

Table 2.3. Challenge times for the three groups of four rams per block.

Group	Start Time (T=-30min.)	Challenge Time (T=0min.)	End Time[a] (T=+120min.)
1	0930 h	1000 h	1200 h
2	1200 h	1230 h	1430 h
3	1430 h	1500 h	1700 h

[a] End time for insulin challenge is 1 hour later (T=+180min.)

Each challenge involved 4 pre-challenge blood samples and 12 post-challenge samples. The times for these blood samples are presented in Table 2.4.

Challenge solutions were administered at time zero and were followed immediately by 6ml of saline. Blood samples (6ml) were taken at the times shown in Table 2.4. The cannulae were then cleared with saline. The presence of this saline in the cannula necessitated the discarding of the first 1ml of blood prior to taking each blood sample. Blood samples were immediately transferred into chilled centrifuge tubes containing sodium citrate (3.5mg/ml of blood) as the anticoagulant. Plasma was separated by centrifugation at 2600 g and 4°C for 20 minutes. The plasma was then pipetted into duplicate vials and stored frozen at -12°C until required for analysis.

Table 2.4 Blood sampling times (minutes) for each challenge

Challenge			
Adrenaline	Insulin	Glucagon	Glucose
-30	-30	-30	-30
-15	-15	-15	-15
-5	-5	-5	-5
-2	-2	-2	-2
Challenges administered at time=0			
2	2.5	2.5	2.5
4	5	5	5
6	7.5	7.5	7.5
8	10	10	10
10	15	15	15
15	20	20	20
20	30	30	30
30	45	45	45
45	60	60	60
60	90	75	75
90	120	90	90
120	180	120	120

(d) Fasting Period

The rams underwent 3 days of fasting, beginning with the removal of feed at 0800 h on day 33. Blood samples were taken twice daily at 0800 h and 2000 h and were processed in the same manner as those samples from the challenge period. The animals were refed at 0800 h on day 36 after the final blood sample was taken. Refeeding was carried out gradually to avoid animals gorging themselves.

3. Determination of Plasma Hormone and Metabolite Concentrations

Samples from all four challenges were assayed for glucose concentration and all but the glucose challenge were assayed for NEFA concentration. Insulin concentration was assayed only in samples from the glucose challenge. The samples taken during the fasting period were analysed for glucose NEFA, insulin, somatotropin, urea and creatinine.

(a) Glucose

Plasma glucose concentrations were determined using a YSI Model 27 industrial analyzer (Yellow Springs Instrument Co., Colorado, USA). This method uses an electrode sensitive to hydrogen peroxide which is produced by the enzymatically catalysed oxidation of glucose. Plasma samples (100ul) are injected into a chamber where they are diluted with buffer and allowed to diffuse through a polycarbonate membrane in which the bound glucose oxidase enzyme is located and where the

oxidation reaction takes place.

The instrument was calibrated using seven glucose standards at concentrations of 0,25,50,75,100,150 and 200 mg/dl. Repeatability of readings was checked by duplicate analysis of the standards after every five samples. The coefficient of variation (CV) between duplicate readings of the standards and samples was less than 1%. The inter-assay CV was 5%.

(b) Non-esterified fatty acids (NEFA)

Plasma NEFA concentrations were determined using an enzymatic colourimetric method (NEFA-C kit, Wako Pure Chemical Industries Ltd., Osaka, Japan). This system is based on enzymatic activation of NEFAs to Coenzyme A esters, which are then oxidised to produce hydrogen peroxide. The presence of peroxidase and hydrogen peroxide allows condensation of 3-methyl-N-ethyl-N-B-hydroxyethyl-analine with 4-amino-antipyrine to form a purple complex, the optical density of which is measured at 550 nm. The standards are provided as oleic acid. The assay methodology was modified according to the procedure of McCutcheon et al. (1986), allowing a reduction of sample and reagent volume, thus reducing assay cost. Assay accuracy was not affected by this modification. Intra-assay and inter-assay coefficients of variation were 3% and 10% respectively.

(c) Urea and creatinine

Plasma urea and creatinine concentrations were determined by an automated method based on the Jaffe Reaction. The plasma samples are dialyzed and divided for the two metabolites to be assayed. From the urea subsample a complex between diacetyl monoxine and urea is produced and thiosemicarbazide and ferric ions are then used to alter and intensify the colour of the complex for reading. (Marsh et al. 1965). The reactions used to generate the colour of the creatinine subsample are described by Chasson et al. (1961). Intra-assay and inter-assay coefficients of variation were 2% and 7% respectively for each metabolite.

(d) Insulin and somatotropin

Insulin and somatatropin were assayed using standard double-antibody competitive binding radioimmunoassays. The assay methodology was described by Flux et al. (1984). Information on the source of the hormone used for standards and trace, the linear range of the assay (log-logit transformed data), and the intra-assay and inter-assay coefficients of variation is presented in Table 2.5.

Table 2.5 Source of hormones and assay parameters for the measurement of insulin and somatotropin concentrations.

	Hormone Source		Assay Parameters		
Assay	Standards	Trace	Linear Range	Intra-Assay CV	Inter-Assay CV
Insulin	Bovine I-5500 Lot 55f-0536[a] 23.4 IU/mg	Bovine I-5500 Lot 55f-0536[a]	100-12800 pg/ml	8.2	12.4
Somato-tropin	Bovine-GH-B1 (AFP 5200[b])	Bovine-GH-I1 (AFP 6500[b])	2-64 ng/ml	8.6	13.2

[a] Sigma Chemical Co., St Louis, Mo., USA.

[b] USDA, Reproduction Laboratory, Beltsville, Md., USA.

(e) Thyroid hormones

Blood plasma samples from the 24 hour collection study were analysed for levels of T_3 and T_4. The 12 two-hourly samples from each animal were pooled into 4 samples, each covering three of the original periods (or 6 hours). The pooled samples were then assayed by radioimmunoassay using TKT35 (for T_3) and TKT45 (for T_4) COAT-A-COUNT^R diagnostic kits (Diagnostic Products Corporation, 5700 West Street.

Los Angeles, Ca. 900045). These involve solid-phase I^{125} radioimmunoassays, based on antibody coated tubes and human serum calibrators. The T_3 and T_4 assay parameters are presented in Table 2.6. All samples for each hormone were run in one assay.

Table 2.6. Assay parameters for the measurement of T_3 and T_4 concentrations.

	T_3	T_4
Linear range	200-600 ng/dl	1.0-24.0 ug/dl
Binding %	38.7	48.8
Intra-assay CV	5.7	7.5

4. Glucose tolerance test

Data arising from the glucose response to the glucose challenge were used to derive the relative disappearance constants (K_g) and the theoretical time zero glucose concentration (G_0). Assuming that the glucose load was instantaneously distributed into one theoretical compartment, the model $G_{(t)}=G_{(0)}.e^{(-tKg)}$ was applied to the glucose response data from 5 to 30 minutes following glucose injection. Glucose distribution space as a percentage of body weight was then calculated from the $G_{(0)}$ concentration and glucose dose rate according to the equation:

$$\% \text{ Space} = \frac{\text{Dose Rate (g/kg)}}{G_{(0)} \text{ (mg/dl)}} \times \frac{100}{1}$$

5. Statistical analysis

Data arising from all sequential blood sampling were subjected to repeated measures analysis using a generalised linear model computer package (REG; Gilmour 1985) to test main effects (line, feeding level, block, sire-within-line and sampling time) and their interactions. Post challenge hormone and metabolite concentrations were corrected for pre-challenge concentrations by subtraction of the mean concentration of the four pre-challenge samples. Statistical analysis (repeated measures) were applied to the pre-challenge data and post-challenge data (both uncorrected and baseline-corrected).

Other parameters, which did not involve sequential sampling, were subjected to analyses of variance to test for the significance of the main effects. Block and sire-within-line effects were rarely significant and have therefore generally not been reported.

Results

1. Liveweight, liveweight gain and backfat measurements

There were no differences in liveweight between lines or feeding levels at the start of the experiment (Table 2.7). Rams offered 0.7 maintenance lost an average of 1.65 kg liveweight during the study. High line rams on the 1.2 maintenance feed level gained an average of 1.2 kg liveweight whereas low line rams at a similar allowance maintained their bodyweight.

Although the two feeding levels were sufficient to induce differences in bodyweight change during the experiment, they did not alter backfat thickness. Thus the only factor to affect backfat thickness at the end of the experiment was selection line ($P<0.01$, Table 2.7). Sire within line had a marginal effect on backfat thickness ($P<0.10$) but no effect on liveweight.

Table 2.7 Weight change and backfat thickness in high backfat line and low backfat line Southdown rams at two feeding levels.

	High backfat line		Low backfat line			Significance[b]		
					Pooled			
Variable	0.7M[a]	1.2M[a]	0.7M	1.2M	SE	L	F	LXF
Weight (kg)[c]	32.7	33.1	32.2	33.1	1.2			
Wt. change (kg)	-1.7	1.2	-1.6	0.1	0.5		***	
Backfat (mm)[d]	2.3	2.4	1.6	1.6	0.3	**		

[a] Feeding level, 0.7 maintenance v. 1.2 maintenance.

[b] Significance of line (L), feeding level (F), effects and their interaction (LXF) based on six rams per cell.

[c] At day 1 of the experiment following a 24 hour fast.

[d] At day 38 of the experiment.

From: Bremmers et al. (1988).

2. Challenge responses

(a) Adrenaline challenge

Plasma concentrations of glucose and NEFA during the intravenous adrenaline challenge (1ug/kg bodyweight) are presented in Figure 2.1. Prechallenge glucose concentrations were stable (no significant time effects) with levels in animals fed 1.2 maintenance being significantly ($P<0.01$) greater than those in animals on 0.7 maintenance. Following the challenge, plasma glucose levels increased rapidly, peaking at 6 minutes post-challenge and then declined,

returning to baseline concentrations by 90 minutes. Post-challenge concentrations, either uncorrected or corrected for baseline, were not significantly influenced by line, feeding level or their interaction.

Baseline NEFA concentrations were significantly ($P<0.01$) greater in animals offered 0.7 maintenance than in those offered 1.2 maintenance and the feeding level by time interaction was also significant ($P<0.05$). Post-challenge NEFA levels rose to peak at 4 to 8 minutes and then declined towards baseline concentration. NEFA levels during the post-challenge period were significantly ($P<0.001$) greater in the 0.7 maintenance feeding group compared with the 1.2 maintenance group for both baseline-corrected and uncorrected data. This reflected a greater lipolytic response (above baseline) to adrenaline in sheep at 0.7 maintenance. The feeding level by time interaction was significant ($P<0.001$) during the post-challenge period. Thus blood NEFA levels in both lines on the 0.7 maintenance feeding level began to increase again from 60 to 120 minutes compared to the 1.2 maintenance group which remained at about baseline NEFA concentration. NEFA concentrations were not influenced by selection line at any period of the challenge.

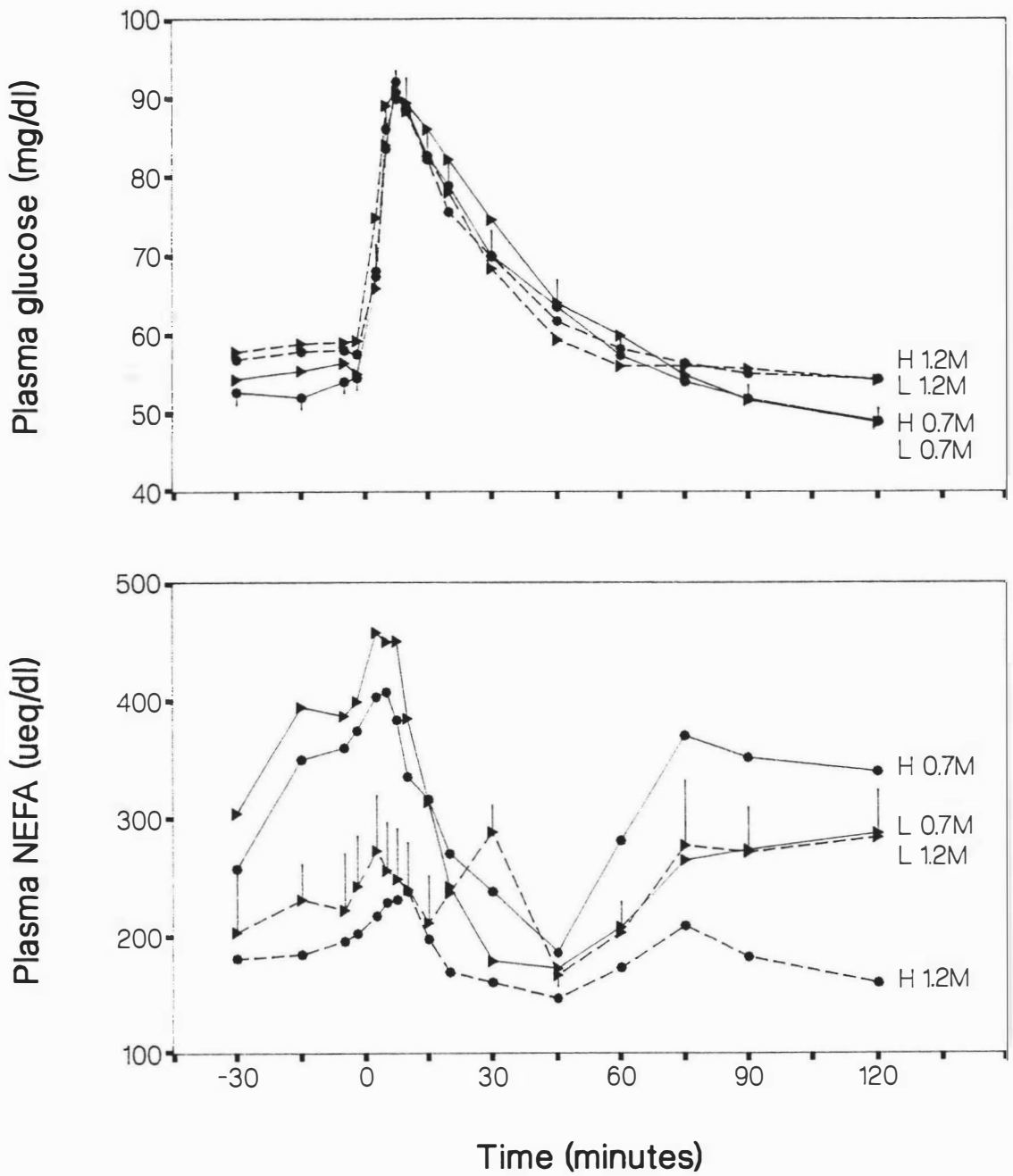

Fig. 2.1 Plasma concentrations of glucose (upper panel) and non-esterified fatty acids (NEFA, lower panel) in 12 high backfat line and 12 low backfat line Southdown rams at 0.7 x maintenance feeding and 1.2 x maintenance feeding during an intravenous adrenaline challenge (1.0ug/kg liveweight administered at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

(b) Insulin challenge

Plasma concentrations of glucose and NEFA during the intravenous insulin challenge (0.01g/kg bodyweight) are presented in Figure 2.2. Prechallenge glucose concentrations were stable (no significant time effect) and a significantly ($P<0.01$) greater baseline glucose concentration was apparent in those animals fed 1.2 maintenance compared with the group fed 0.7 maintenance. The low backfat selection line tended ($P<0.10$) to have a greater baseline glucose concentration than the high backfat animals but differences in glucose concentration became non-significant during the post-challenge period. Plasma glucose concentration decreased rapidly in the first 45 to 60 minutes following the infusion of insulin and then increased towards baseline values from 60 to 180 minutes. Post-challenge glucose was significantly ($P<0.001$) lower in the 1.2 maintenance-fed animals than in those on 0.7 maintenance, when corrected for prechallenge levels, but was nonsignificant in the uncorrected data. This baseline-corrected difference reflects the greater sensitivity of the 1.2 maintenance group to insulin compared with the 0.7 maintenance group. The line by time interaction was significant ($P<0.05$) in post-challenge glucose concentrations, both corrected and uncorrected for prechallenge concentrations. This line by time relationship can be seen in Figure 2.3. where glucose concentrations of each selection line are plotted against time. The corrected glucose response to insulin in the low backfat line stayed depressed for longer than glucose concentration in the high backfat line.

The plasma NEFA response to insulin involved an initial decrease in

NEFA concentration, during 2.5 to 30 minutes post-challenge, in response to endogenous glucagon secretion. This was then followed by an increase in NEFA concentration peaking at between 60 and 120 minutes post-challenge before dropping towards baseline level. Basal and post-challenge NEFA concentrations were significantly ($P<0.01$ and $P<0.05$) greater in the animals fed 0.7 maintenance than those fed 1.2 maintenance. Post-challenge differences in NEFA concentrations became nonsignificant when corrected for prechallenge NEFA levels. The post-challenge feeding level by time interaction was significant ($P<0.001$) for both the baseline-corrected and uncorrected NEFA concentrations.

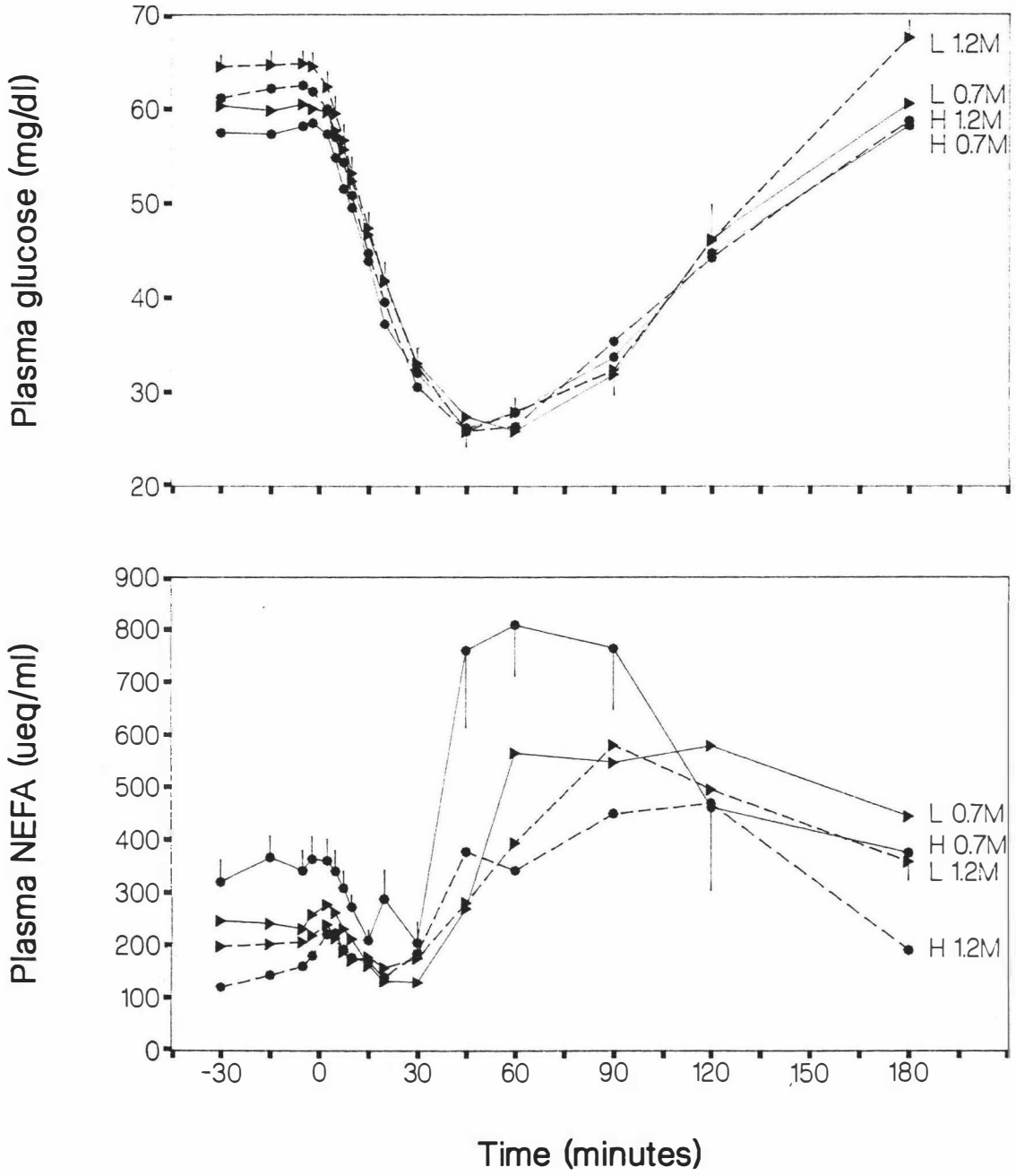

Fig. 2.2 Plasma concentrations of glucose (upper panel) and non-esterified fatty acids (NEFA, lower panel) in 12 high backfat line and 12 low backfat line Southdown rams at 0.7 x maintenance feeding and 1.2 x maintenance feeding during an intravenous insulin challenge (0.01g/kg liveweight administered at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

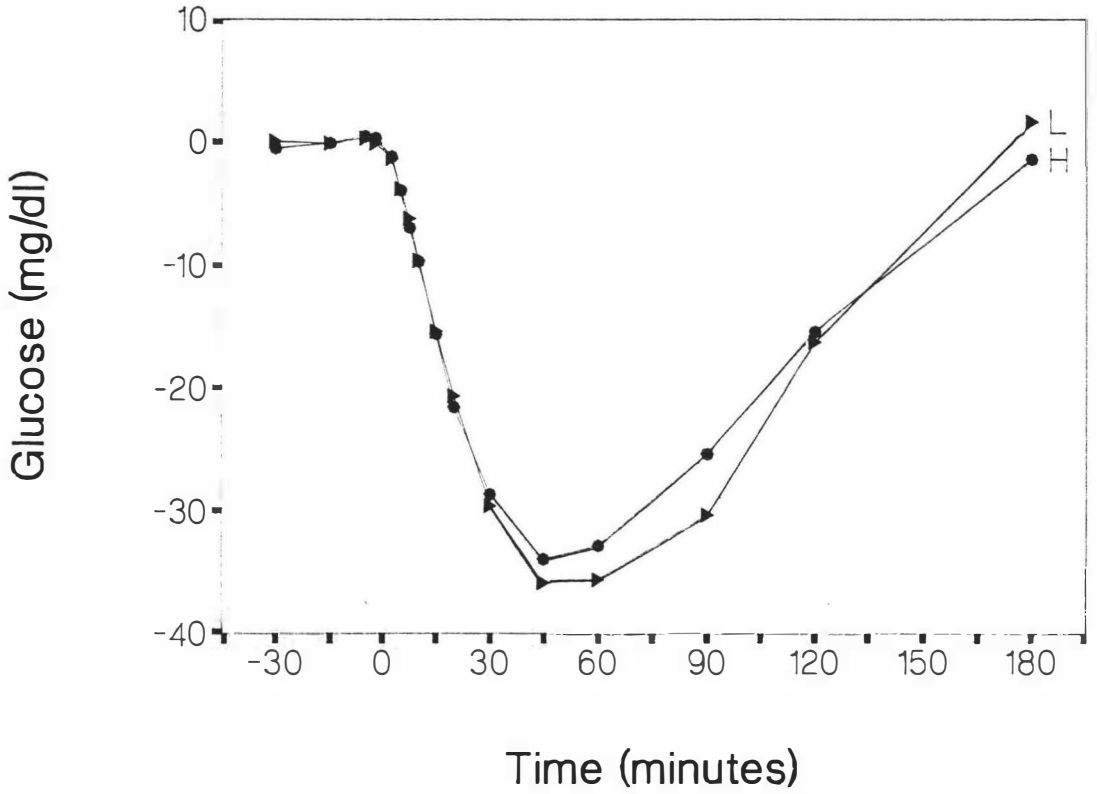

Fig. 2.3 Plasma concentrations of glucose, corrected for pre-challenge glucose concentration, in 12 high backfat line and 12 low backfat line Southdown rams during an intravenous insulin challenge (0.17g/kg liveweight administered at time zero).

High backfat line
Low backfat line

(c) Glucose challenge

Plasma concentrations of glucose and insulin during the intravenous glucose challenge (0.17g/kg bodyweight) are presented in Figure 2.4. Plasma glucose rose to a maximum concentration within the first 2.5 minutes post-challenge, returning to baseline by 120 minutes. Prechallenge and post-challenge (uncorrected or corrected) glucose concentrations were not significantly influenced by selection line, feeding level or their interaction. The theoretical $G_{(0)}$ levels, clearance rate and distribution space values for the glucose response above baseline levels are presented in Table 2.8. The K_g values for the 1.2 maintenance-fed animals were significantly ($P<0.001$) greater thanthose of the 0.7 maintenance-fed animals, indicating a more rapid clearance of infused glucose in the well fed animals. The glucose concentration above baseline calculated for Time=0 ($G_{(0)}$) did not differ between the selection lines or feeding levels.

Table 2.8 Glucose tolerance test parameters after an intravenous glucose challenge (1.7g/kg liveweight) in high backfat line or low backfat line Southdown rams on 2 feeding levels.

	High backfat line		Low backfat line			Significance[b]		
					Pooled			
Variable	0.7M[a]	1.2M[a]	0.7M	1.2M	SE[c]	L	F	LXF
$G_{(0)}$[d] (mg/dl)	99.9	102.6	95.6	101.7	3.9			
K_g[e] (min^{-1})	-0.026	-0.040	-0.025	-0.045	0.004		***	
Space[f] (%)	17.1	16.8	17.9	16.9	0.7			

[a] feeding level, 0.7 maintenance v. 1.2 maintenance

[b] significance of line (L), feeding level (F), effects and their interaction (LXF) based on six rams per cell

[c] pooled standard error

[d] Post-challenge glucose concentration extrapolated back to time=0

[e] rate of glucose disappearence

[f] glucose distribution space

Baseline insulin concentrations were significantly ($P<0.001$) greater in animals offered 1.2 maintenance than in those offered 0.7 maintenance. The line by feeding level interaction in baseline concentrations was also significant ($P<0.05$). High backfat line animals fed 1.2 maintenance maintained substantially greater plasma insulin levels than animals fed 0.7 maintenance. However in the low backfat line, insulin was dependent on feeding level. Plasma insulin

levels rose rapidly in response to the glucose challenge and peaked at between 7.5 and 10 minutes and then returned gradually to baseline values by 90 to 120 minutes. Post-challenge insulin concentrations were influenced ($P<0.05$) by feeding level but differences became nonsignificant when corrected for prechallenge insulin concentration. The post-challenge feeding level by time interaction was also significant ($P<0.05$).

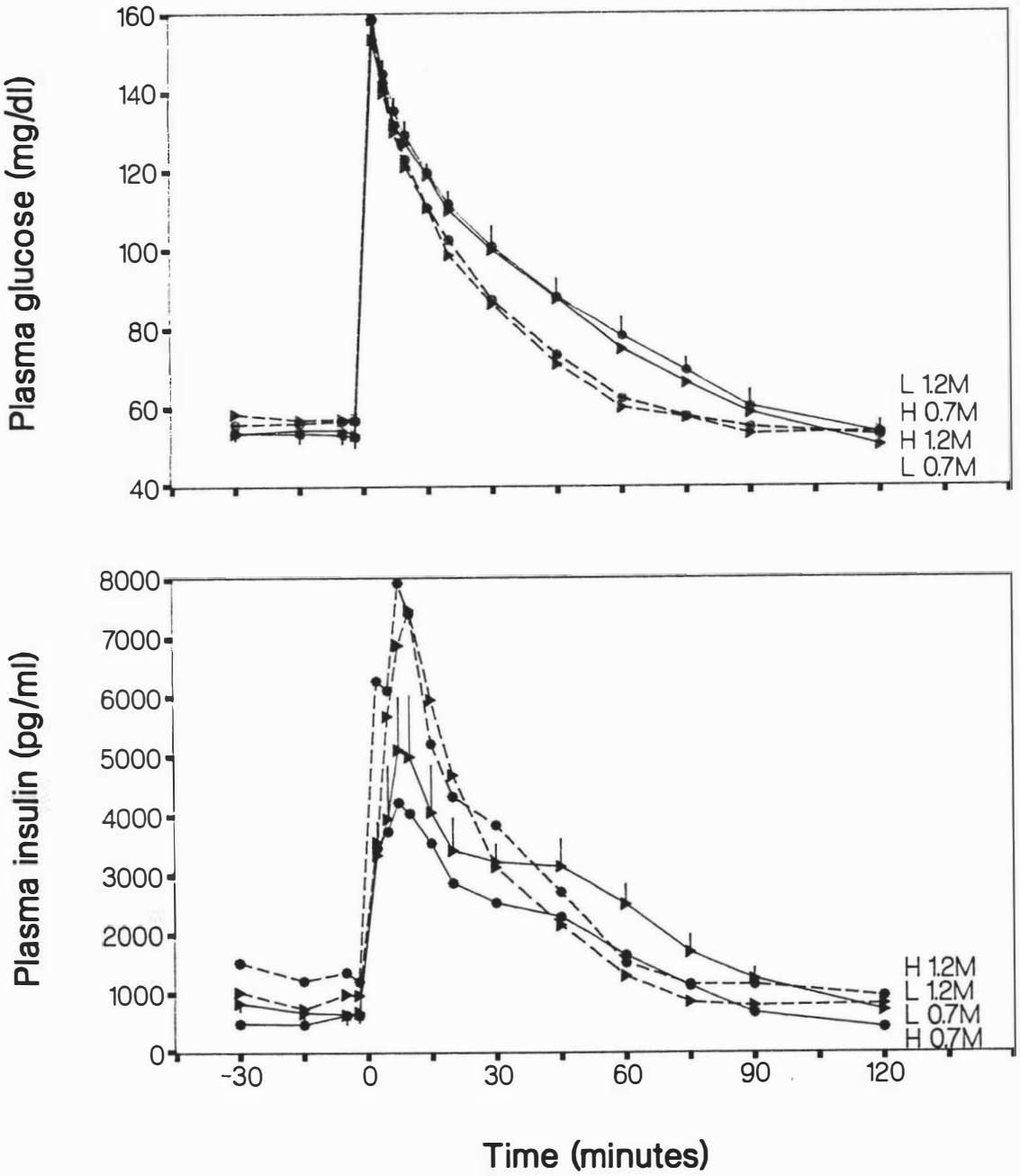

Fig. 2.4 Plasma concentrations of glucose (upper panel) and insulin (lower panel) in 12 high backfat line and 12 low backfat line Southdown rams at 0.7 x maintenance feeding and 1.2 x maintenance feeding during an intravenous glucose challenge (0.17g/kg liveweight administered at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

(d) Glucagon challenge

Plasma concentrations of glucose and NEFA during the intravenous glucagon challenge (0.175ug/kg bodyweight) are presented in Figure 2.5. Baseline glucose concentrations were stable (no significant time effects) and levels in the animals offered 1.2 maintenance were significantly ($P<0.01$) greater than those in rams offered 0.7 maintenance. The post-challenge glucose levels rose rapidly, peaking at 7.5 minutes and returned to baseline levels by 75 to 120 minutes. The post-challenge glucose levels (corrected for baseline concentration) tended ($P<0.1$) to be higher for animals offered 0.7 maintenance than those offered 1.2 maintenance. Glucose concentration following the glucagon challenge was not significantly influenced by selection line or the interaction with feeding level.

Basal NEFA concentrations were significantly ($P<0.01$) greater in the 0.7 maintenance-fed animals than in those fed 1.2 maintenance. Post-challenge NEFA levels increased, reaching a maximum between 2.5 and 10 minutes after glucagon infusion, dropped below baseline level by 45 minutes and then returned to baseline concentration by 75 minutes. Post-challenge NEFA levels in the group fed 0.7 maintenance were significantly ($P<0.001$) greater than those fed at 1.2 maintenance. When post-challenge NEFA response was corrected for prechallenge NEFA concentration, the group fed 0.7 maintenance was significantly ($P<0.05$) lower than the 1.2 maintenance group. The feed by time interaction was significant ($P<0.001$) for the post-challenge period, also reflecting the difference between feeding levels in response to the glucagon challenge.

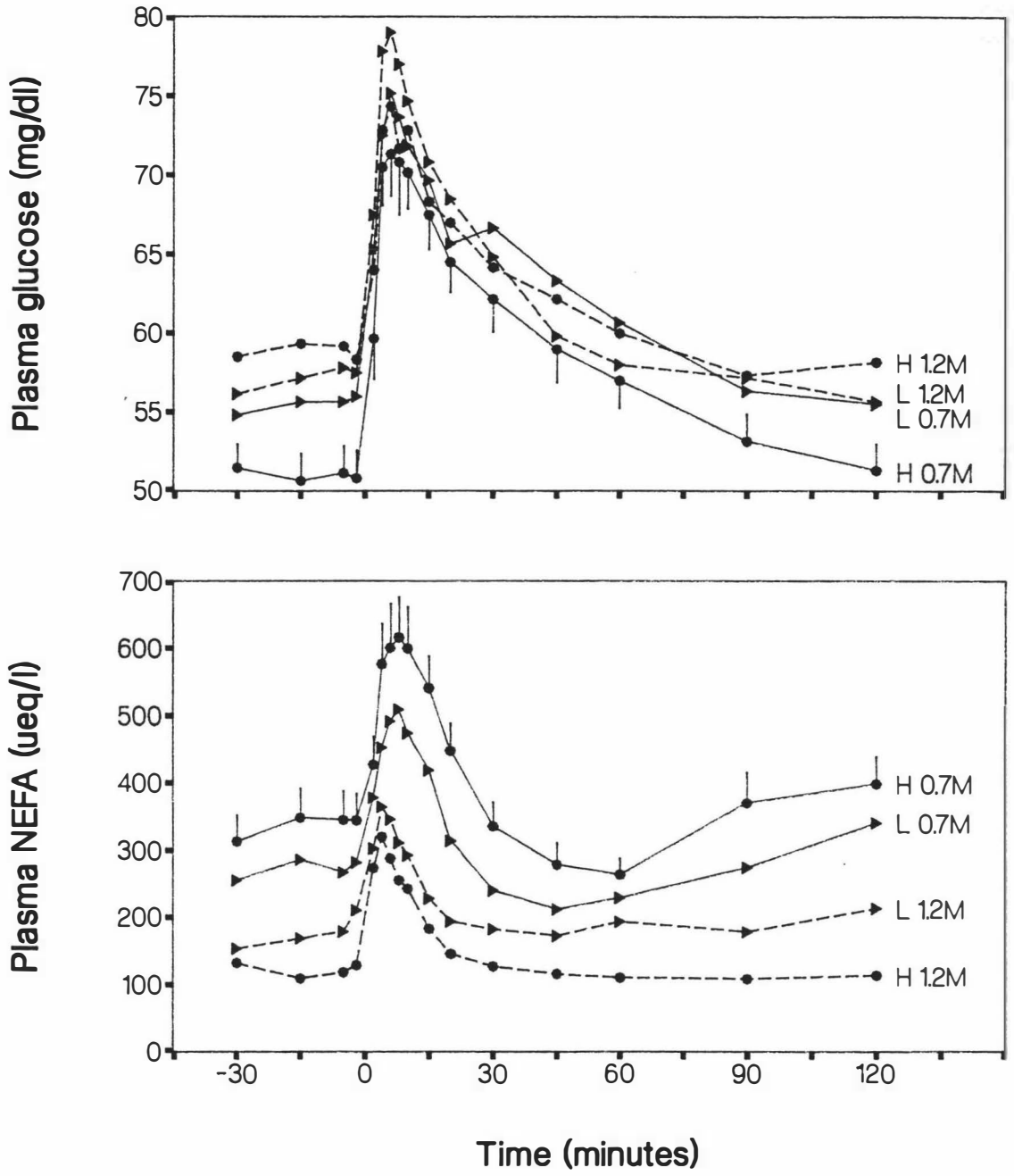

Fig. 2.5 Plasma concentrations of glucose (upper panel) and non-esterified fatty acids (NEFA, lower panel) in 12 high backfat line and 12 low backfat line Southdown rams at 0.7 x maintenance feeding and 1.2 x maintenance feeding during an intravenous glucagon challenge (0.175ug/kg liveweight administered at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
——▶—— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

3. Fasting responses

The plasma concentrations of glucose, NEFA, insulin, somatotropin, urea and creatinine, in response to the 72 hour fast, are presented in Figure 2.6 to 2.8.

Plasma glucose and insulin concentration decreased in response to fasting and reached constant levels by 48 hours. Plasma NEFA levels increased over the first 48 hours of the fast and remained at a maximum for the remaining 24 hours. The fasting concentrations of glucose, insulin and NEFA were not significantly influenced by line, feeding level or their interaction.

Fasting somatotropin levels were significantly greater in the low backfat line ($P<0.05$) and in those animals that were fed at 1.2 maintenance ($P<0.01$). No interactions between line and feeding level were apparent for the fasting somatotropin response.

Plasma urea concentration decreased from 12 to 72 hours of the fasting period. Animals offered 1.2 maintenance had significantly ($P<0.01$) greater plasma urea levels than those offered 0.7 maintenance and the feed level by time interaction was also significant ($P<0.001$) reflecting a convergence between the feeding levels as the fast proceeded. No line effects were apparent for the plasma urea response to fasting.

Rams offered 0.7 maintenance had a significantly ($P<0.01$) greater plasma creatinine level than those offered 1.2 maintenance. This

result is consistent with the greater rate of degradation of muscle protein associated with chronic underfeeding.

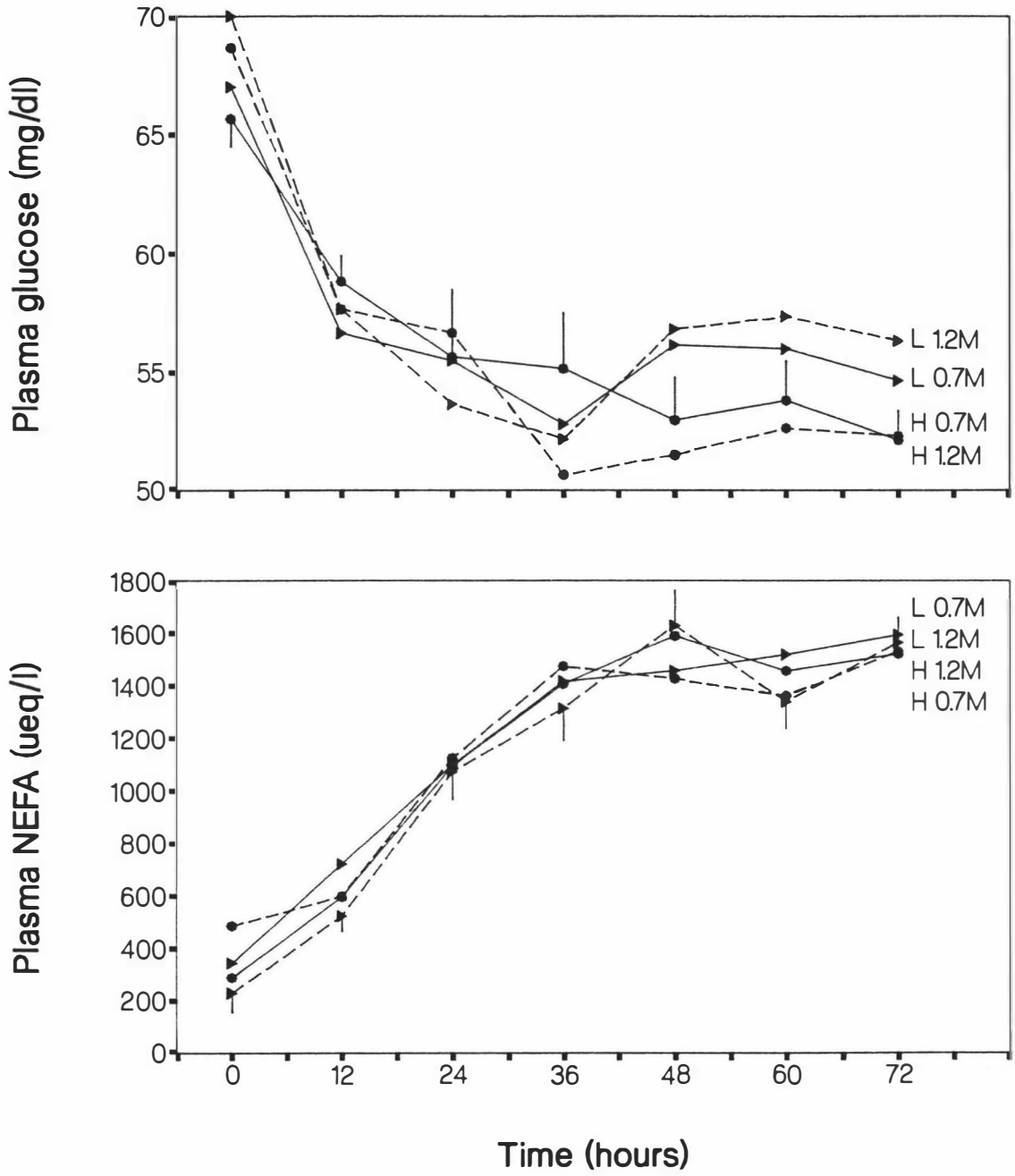

Fig. 2.6 Plasma concentrations of glucose (upper panel) and non-esterified fatty acids (NEFA, lower panel) in 12 high backfat line and 12 low backfat line Southdown rams (previously offered 0.7 x maintenance feeding and 1.2 x maintenance feeding) during a 72 hour fast (beginning at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

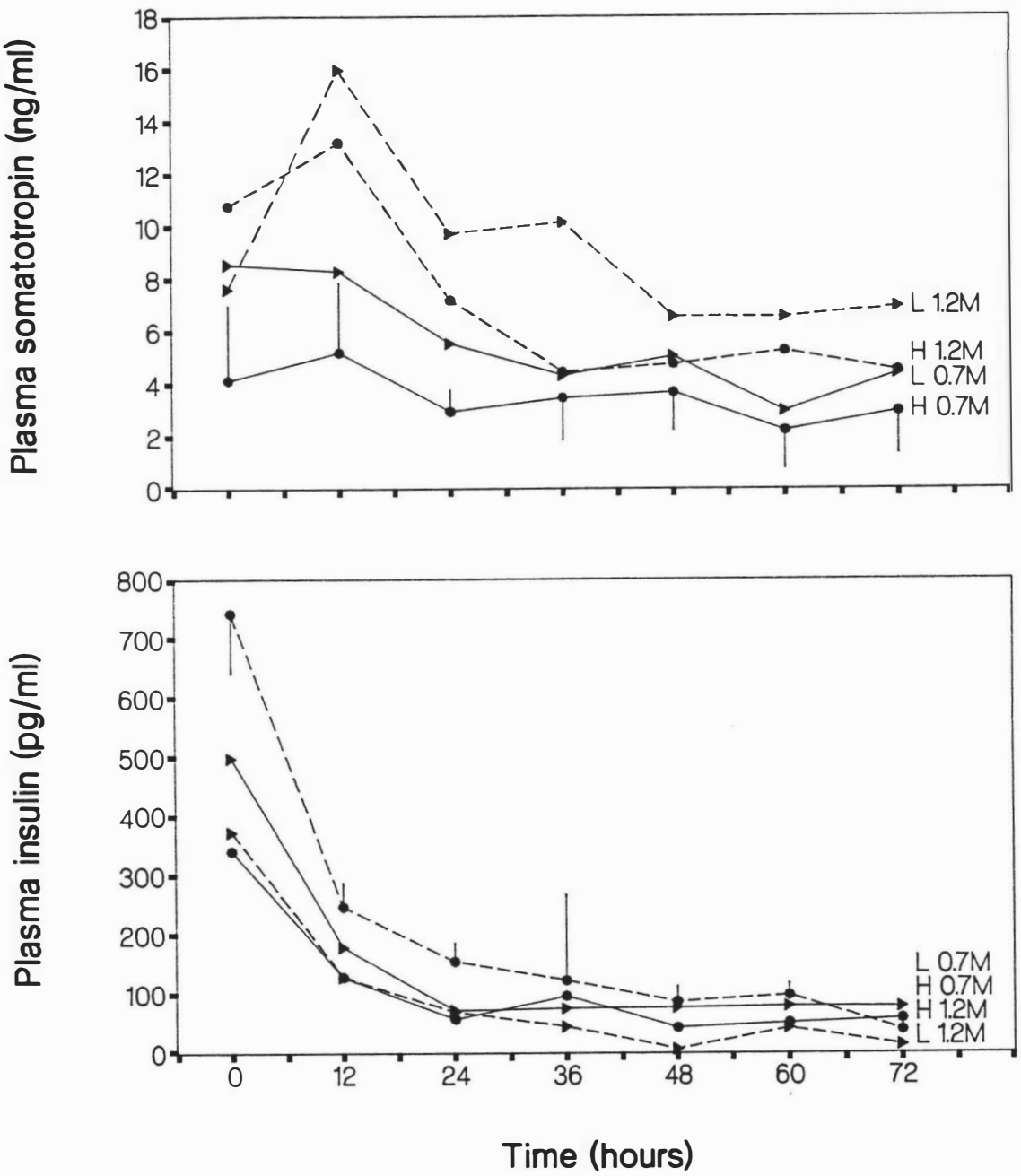

Fig. 2.7 Plasma concentrations of somatotropin (upper panel) and insulin (lower panel) in 12 high backfat line and 12 low backfat line Southdown rams (previously offered 0.7 x maintenance feeding and 1.2 x maintenance feeding) during a 72 hour fast (beginning at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
——▶—— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

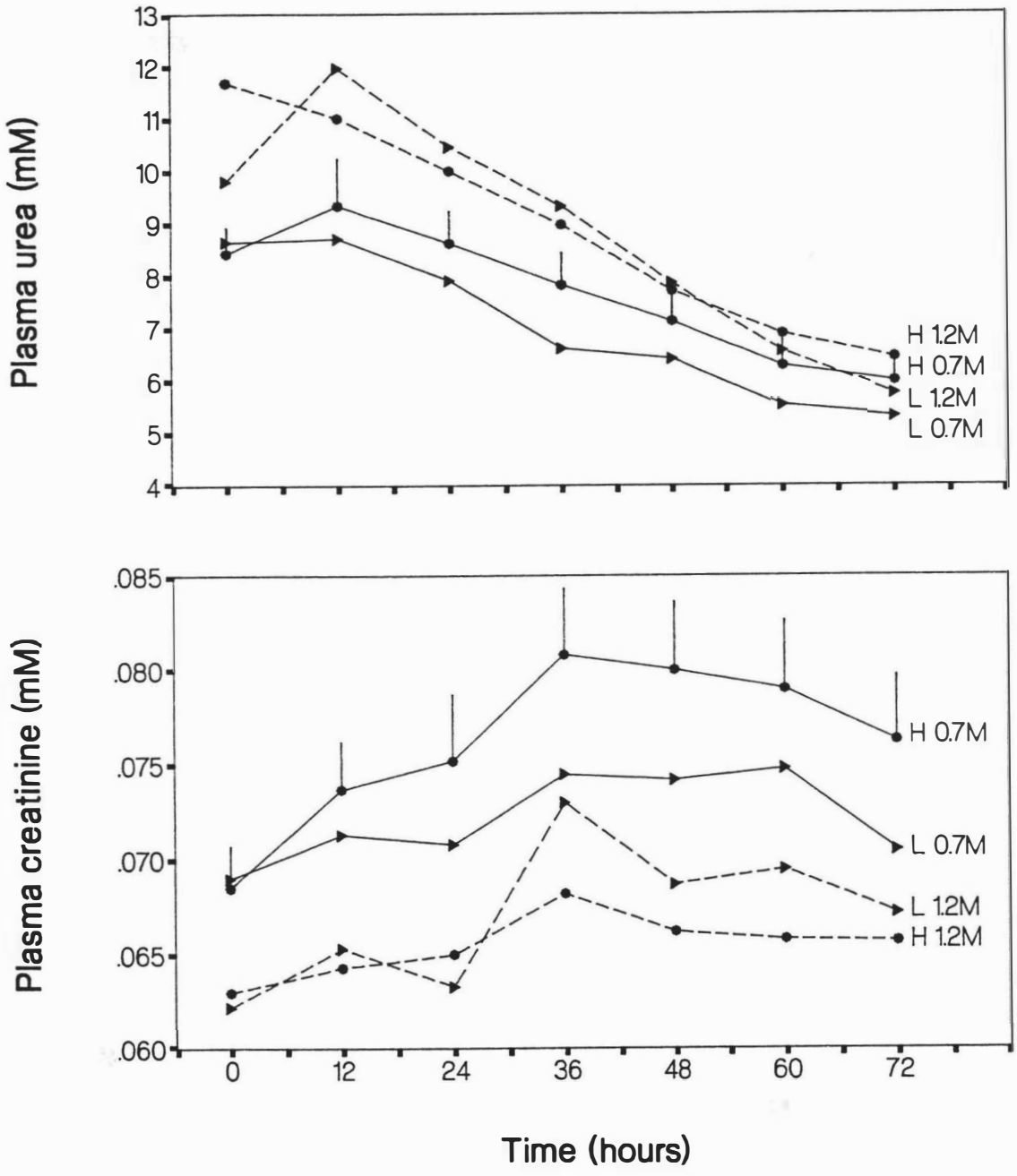

Fig. 2.8 Plasma concentrations of urea (upper panel) and creatinine (lower panel) in 12 high backfat line and 12 low backfat line Southdown rams (previously offered 0.7 x maintenance feeding and 1.2 x maintenance feeding) during a 72 hour fast (beginning at time zero). Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

4. Thyroid Hormones

The pooled plasma concentrations of T_3 and T_4 from the diurnal study of Bremmers et al. (1988) are presented in Figure 2.9. Plasma T_3 concentration in the low backfat selection line group was significantly ($P<0.001$) greater than in the high line and the line by time interaction was significant ($P<0.001$). Plasma T_4 was not affected by line but the line by time interaction was again significant ($P<0.01$).

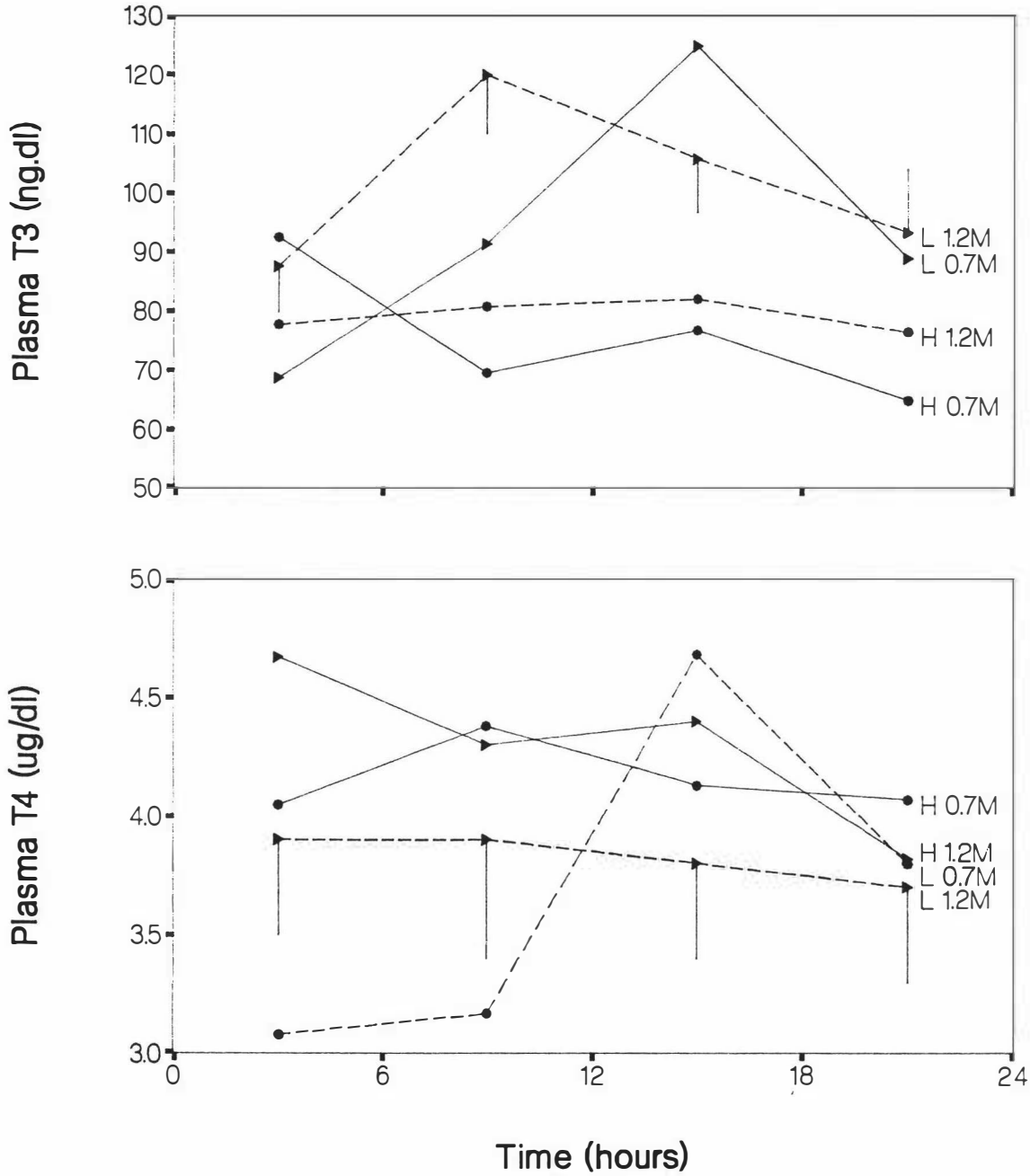

Fig. 2.9 Plasma concentrations of triiodothyronine (T$3, upper panel) and thyroxine (T$4, lower panel) in 12 high backfat line and 12 low backfat line Southdown rams fed every 2 hours at 0.7 x maintenance feeding and 1.2 x maintenance feeding during 24 hours (beginning at time zero). Samples were the pooled over 6 hour periods. Vertical bars denote the pooled standard error.

—●— High backfat line, 0.7 M feed level (H 0.7M)
– –●– – High backfat line, 1.2 M feed level (H 1.2M)
—▶— Low backfat line, 0.7 M feed level (L 0.7M)
– –▶– – Low backfat line, 1.2 M feed level (L 1.2M)

DISCUSSION

The divergent feeding levels used in this study were successful in creating differences in physiological responses to metabolic challenges. Rams on the 0.7 maintenance allowance had elevated basal NEFA concentrations and a greater lipolytic response to the glucagon and adrenaline challenges compared with those on 1.2 maintenance. These differences in fatty acid metabolism are commonly found in underfed animals as fat stores are mobilised and catabolised so as to conserve limited glucose stores (Ganong 1981; Rule et al. 1985).

Baseline (prechallenge) glucose and insulin concentrations were greater in rams on the 1.2 maintenance feeding level as was also found in the same animals by Bremmers et al. (1988). The rate at which plasma glucose was cleared after the glucose challenge was greater in the rams fed the 1.2 maintenance allowance than in those fed at 0.7 maintenance. In addition, rams fed 1.2 times maintenance cleared glucose more rapidly in response to exogenous insulin than those fed 0.7 maintenance. There was, however, no difference between feeding levels in insulin response to the glucose challenge. This would suggest that a difference exists between the feeding levels in sensitivity to circulating insulin but not in pancreatic sensitivity to blood glucose. The number and affinity of insulin receptors in tissue is known to be influenced by the level of nutrition (Ganong 1981) and this may account for the greater sensitivity to insulin of the well fed animals in this study.

The fasting challenge generated differences between the feeding levels in plasma concentrations of urea and creatinine. Animals previously offered the 0.7 maintenance allowance had lower urea and higher creatinine levels than those animals offered 1.2 maintenance. These differences appeared to reflect differences which already existed at the beginning of the fast. Thus the level of urea in the two feeding level groups converged as the fast proceeded, presumably due to digesta being progressively removed from the rumen of the high feeding level animals. Creatinine levels were parallel in the two feeding level groups reflecting greater muscle breakdown in the animals which had been on 0.7 time maintenance allowance.

The high and low backfat selection lines used in this study displayed several physiological differences. The low backfat animals had a greater reduction in plasma glucose levels in response to the insulin challenge. No between-line differences were apparent in the insulin response to the glucose challenge or in rate of glucose clearance following the glucose challenge.

Thus between-line differences in glucose clearance were apparent when exogenous insulin was administered but not when endogenous insulin was elevated in response to a glucose load. Administration of the glucose challenge elevated circulating insulin to between 4 and 8 ng/ml (Figure 2.4). Land et al. (1983) administered approximately 8 ug/kg insulin to calves (ie. 0.2 units/kg assuming insulin has 26 units/mg) and found that insulin levels were elevated to 110 ng/ml. Thus the dose of insulin used in the present study (10 ug/kg) would likely have increased circulating insulin levels by a similar amount

(ie. 14 to 28 times the peak in endogenous insulin produced by the glucose challenge). This suggests that the between line difference in glucose clearance was evident when pharmecological doses of insulin were used but not when high physiological levels were attained by the glucse challenge. Carter et al. (1989) were unable to find any differences between the Southdown selection lines in glucose-insulin status other than those accounted for by differences in the glucose distribution space. Lord et al. (1985) were also unable to find any glucose-insulin status differences between lines of Coopworth sheep selected on the basis of body composition.

There were no line differences in the lipolytic response to the glucagon challenge. Carter et al. (1989) found a greater NEFA response to a glucagon challenge in the low backfat selection line animals. Greater lipolytic responses to adrenaline challenges have been found in lean pig selection lines (Standal et al. 1973, 1979; Mersmann 1985; Brocklen et al. 1986) compared with pigs from fat lines. No such differences in lipolytic response to adrenaline challenges have been found in the Southdown selection lines.

The low backfat line rams had greater fasting levels of circulating somatotropin compared with the high backfat line. Carter et al. (1989) found similar differences in somatotropin levels during a fast and subsequent refeeding period in ram lambs from the same selection lines. The finding of greater levels of somatotropin in the low backfat sheep line is consistent with results showing higher levels of somatotropin in lean, fast growing pig selection lines compared with contemporary fat, slow growing genotypes (Ringberg Lund-Larsen et al., 1975; Althen et al., 1976; Wangsness et al. 1977). However a more

recent experiment involving the Southdown sheep selection lines carried out by Van Maanen et al. (1989) failed to detect any line differences in fasting levels of somatotropin. There are difficulties in assessing the somatotropin status of animals as the hormone is secreted from the pituitary gland in pulses (Klindt et al. 1985). Intensive blood sampling is required to monitor these pulses and the inconsistent results found in the Southdown lines may reflect the use of an inadequate sampling pattern.

There were no differences in fasting levels of urea between the selection lines. This contrasts with the lower fasting urea levels previously found in the low backfat selection line (Carter et al. 1989; Van Maanen et al. 1989).

Basal circulating T_3 concentration was greater in the low backfat animals during the 24 hour basal study. Lean selection line pigs have also displayed greater T_3 concentrations (Bakke and Tveit, 1977; Standal et al. 1980; Yen et al. 1985). These differences in T_3 levels may reflect differences in maintenance requirements of the selection lines used in these studies. The similarity between results from this study and those from the pig selection lines suggests that thyroid hormone status of the Southdown sheep requires further investigation. As a first approach, studies should examine more thoroughly the circulating levels of thyroid hormones and thyroid hormone release in response to a challenge of thyroid stimulating hormone (TSH) or thyrotropin releasing hormone (TRH).

In conclusion the divergent feeding levels used in this study were successful in creating differences in physiological responses to metabolic challenges, but no feeding level by selection line differences were apparent. There may be differences in the maintenance requirements of the selection lines and therefore in energy balance. This would complicate the calculation of standardised conditions necessary for the identification of potential genetic markers. Whatever the reason for inconsistent results from successive studies of these lines, they call into question the possible use of metabolic parameters to predict genetic merit for lean tissue growth.

CHAPTER III

GENERAL DISCUSSION

In the underfed or fasted animal metabolic compensation occurs in an attempt to maintain essential physiological processes. Associated with underfeeding there is a drop in the circulating levels of catecholamines and a reduction in sympathetic nervous function. Basal metabolic rate is depressed and this is largely responsible for the reduction in the rate of weight loss seen after prolonged fasting. Body stores of nutrients are mobilised and there are changes in the levels of regulating hormones and in tissue sensitivity to these hormones. These changes ensure that the low priority tissues (eg. adipose) are depleted first. Examples of compensatory mechanisms have been seen in the responses to underfeeding and fasting in this experiment. Basal blood glucose and insulin levels were depressed in the 0.7 maintenance fed group reflecting the lower amount of stored glucose and the attempt to maintain blood glucose levels respectively. Glucose is stored in the form of glycogen in the hepatic, and to a lesser extent peripheral, tissues of the body. Glycogen serves only to act as a buffer between glucose supply from meals and demand as a substrate for metabolic processes. Synthesis of glycogen from glucose is stimulated by insulin. Insulin receptor numbers and affinity are nutritionally dependant and thus the underfed animals in this study had a lower rate of glucose clearance in response to the insulin challenge. Mobilisation of glycogen is stimulated by glucagon, the level of which decreases as carbohydrate stores are reduced and fatty acids and ketones become the major energy source. The low feeding

level groups also had elevated NEFA concentrations reflecting the increased mobilisation of stored lipids. Fat stores are mobilised by B-oxidation, releasing acetyl-CoA which is converted into ketones by the liver. The ketones then circulate to other tissues where they enter the TCA cycle to liberate energy. The rate of lipolysis is increased by somatotropin, glucocorticoids, thyroid hormones, glucagon and catacholamines via the activation of hormone-sensitive lipase. The level of plasma NEFA was elevated in response to the fast, the glucagon challenge and to the insulin challenge due to endogenous glucagon secretion. When stores of fat become low in the ronically underfed animal, protein is catabolised. Liver, spleen and muscle tissues are the first protein stores to be catabolised, yeilding amino acids which can enter glycogenic pathways. Blood creatinine levels indicate the rate of protein degradation and were greater in the underfed animals.

Despite the many physiological differences generated by the two feeding levels in this study, no interactions between energy balance and animal genotype were apparent. Given what is known about the physiological differences between other lines of animals selected on the basis of body composition, it was expected that the Southdown selection lines used in this study would have shown differences in response to metabolic challenges and that the magnitude of these differences might have been influenced by level of nutrition. A possible explanation for the absence of a genotype by energy allowance interaction is that the true maintenance requirements of the selection lines may differ and therefore that the calculated feed allocations did not result in comparisons being made at equal energy balances.

Maintenance energy requirement is the energy cost of maintaining essential body functions and a constant body temperature. It is often measured using calorimetry by monitoring the basal metabolic rate (BMR) of the animal. BMR is a function of surface area to volume ratio and is related exponentially to body weight. Thus an expression of liveweight on a "metabolic" basis is often used to calculate maintenance energy requirement. Liveweight raised to the power of 0.75 has been derived empirically and is most commonly used to estimate metabolic liveweight. However this exponent has been found to vary considerably (0.4-0.85) between animals in studies of metabolic efficiency (Baldwin et al. 1984). Furthermore there is evidence with sheep and other species that body composition affects maintenance requirement, due to the relatively low metabolic activity of adipose tissue (Graham 1967). Thus the maintenance requirement of a fat sheep may be lower than that of a lean sheep when compared at the same weight. The measurements of ultrasonic backfat depth in the sheep used in the present study became significantly different by the end of the trial. Hence maintenance requirement of animals from the high backfat selection line could have been lower than that of the lean animals during the trial.

In a review of studies on nutritional energetics in animals, Baldwin et al. (1984) showed strong positive correlations between BMR and all relative weights of visceral organs and the gastrointestinal tract. The energy expenditure of these tissues appears to account for a substantial proportion of total maintenance requirements and is mainly related to the synthesis of protein in these organs. Yen et al. (1983) showed by indirect calorimetry that a fat selection line of

pigs had a lower BMR than a contemporary lean selection line. Lower relative weights of gut and visceral organs in the fat pigs were thought to explain this difference. In experiments with rams drawn from the Massey University high and low backfat selection lines the low backfat animals consistently showed higher visceral organ weights (Kadim et al. 1989). However differences were small (approximately 5%) and were not always significant.

Higher circulating levels of T_3 were found in the low backfat line rams in the present study. This is consistent with observations in lean pig selection lines compared with the fat lines (Bakke and Tveit, 1977; Standal et al. 1980; Yen et al. 1985). York et al. (1978) have shown similar differences in plasma levels of T_3 in lean and obese mice. T_3 is the more active form of thyroid hormone and has a stimulatory effect on heat production. Plasma T_3 differences might also contribute to line differences in basal metabolic rate and therefore in maintenance requirements of animals.

Differences in body fat content, visceral organ weight and circulating T_3 concentration could therefore have been responsible for a lower maintenance requirement in the high backfat selection line animals used in the present study. If this were so, energy balance in the high backfat animals would have been greater than that intended by the allocation to assumed 0.7 and 1.2 times maintenance rations. The consequences of this may be examined in relation to the energy balance data collected in the previous experiment on the same animals (Bremmers et al. 1988). Table 3.1 shows the amounts of energy retained by the two lines on each feeding level. Net energy retention

has been calculated for all four groups assuming that the high backfat animals have a lower maintenance requirement by 0%, 10% or 20% compared with low backfat rams.

Table 3.1 Liveweight change and calculated net energy retention at different maintenance requirements for high backfat line and low backfat line Southdown rams at two feeding levels.

	High BF line		Low BF line		High BF - Low BF	
Variable	0.7M[a]	1.2M[a]	0.7M	1.2M	0.7M	1.2M
Wt. change (kg)[b]	-1.7	1.2	-1.6	0.1		
Retained energy[c] (Mj/d)	6.5	11.3	6.4	9.9		
Net retention[d]	-3.1	1.6	-3.3	0.2	0.2	1.4
" " (-10%)[e]	-2.1	2.6	-3.3	0.2	1.2	2.4
" " (-20%)[e]	-1.2	3.6	-3.3	0.2	2.2	3.4

[a] Feeding level, 0.7 maintenance v. 1.2 maintenance.

[b] Weight change over the 38 days of the experiment.

[c] Retention=energy intake - (urine and faecal) losses.

[d] Net retention=retained energy corrected for maintenance (assuming maintenance requirement is equal in the two lines).

[e] Net retention (assuming maintenance requirement was 10% or 20% higher in the high backfat line).

Adapted from: Bremmers et al. (1988).

Thus if such differences in maintenance energy requirements did

exist, considerable differences would have been generated in the actual energy balances of animals in each selection line for each feed level. The levels of energy retention of the high and low lines fed at 0.7 times assumed maintenance requirement were similar. However, the low line animals offered the 1.2 maintenance diet had a lower level of energy retention than the high line animals on the same plane of nutrition. This was a result of greater selectivity among the low line rams who frequently failed to consume all the lucerne, particularly the stalk. Thus diet selectivity of one line contributed to a difference between the energy balance intended and that actually achieved.

If, due to differences in maintenance requirements, the high line rams were on a more positive energy balance than the low line rams, they would be expected to have greater levels of glucose and insulin, and lower levels of NEFA and creatinine (Ganong 1981; Rule et al. 1985). The between line differences did involve greater levels of insulin in the high line but also involved lower glucose concentrations, and higher levels of creatinine, with no differences in NEFA concentrations. Thus, if a difference in maintenance requirement, and therefore energy balance, did exist in the present study, it was not clearly seen in basal concentrations of hormones and metabolites. Nevertheless, there appears to be some evidence that the lines differ in maintenance requirement. This should be examined directly by calorimetry techniques so that more precise allocation of feeding levels may be undertaken in future studies.

A number of physiological differences have been found in

comparisons of the Massey University Southdown selection lines. These differences represent potential markers of genetic merit. However a major problem with the findings to date is that differences have not been consistently expressed. Differences in fasting levels of somatotropin have been found between these selection lines in this study and by Carter et al. (1989) but not in a larger study by van Maanen et al. (1989). Differences in the glucose/insulin status and in the lipolytic responses to challenges with adrenaline or glucagon have also been inconsistent. Plasma urea differences between lines have been the most reliable result to date. However urea differences are diminished at low feeding levels (Bremmers et al. 1988) and in field grazing conditions (McCutcheon et al. 1987).

In conclusion, the results of studies so far undertaken with the Massey University Southdown selection lines are equivocal with respect to the development of markers for lean growth. While some differences between the lines have been observed, they have not been repeatable between studies. The results of this study suggest that the lack of repeatability was not due to failure to control energy status of the animals since few line by feeding level interactions were apparent. However, this conclusion must be seen in light of previous comments concerning possible differences between the lines in maintenance energy requirements. It may be that the small number of sires used in each line is responsible for the lack of consistent differences because, with two sires per line in each generation, any one sire has the potential to disproportionately influence "between-line" differences in his progeny. However, the size of selection lines is generally limited by economic constraints. Thus a better approach to

investigating genetic markers may be to measure metabolic characteristics in a large number of randomly-selected sires and then estimate their genetic merit for lean tissue growth directly by progeny testing.

APPENDIX I

Arrangement of rams in the metabolism crates and their line, feed level, block and sample group.

	TAG	SIRE	LINE[a]	FL[b]	TAG	SIRE	LINE	FL
BLOCK 1 GROUP[c]								
1	83	4	LOW	0.7M	18	1	HIGH	1.2M
	143	3	LOW	1.2M	16	2	HIGH	0.7M
2	31	4	LOW	0.7M	92	2	HIGH	1.2M
	89	3	LOW	1.2M	103	1	HIGH	0.7M
3	41	3	LOW	0.7M	65	2	HIGH	1.2M
	150	4	LOW	1.2M	55	1	HIGH	0.7M
BLOCK 2 GROUP								
1	50	4	LOW	0.7M	24	1	HIGH	1.2M
	114	3	LOW	1.2M	189	2	HIGH	0.7M
2	62	4	LOW	0.7M	95	1	HIGH	1.2M
	2	3	LOW	1.2M	117	2	HIGH	0.7M
3	56	3	LOW	0.7M	147	2	HIGH	1.2M
	105	4	LOW	1.2M	85	1	HIGH	0.7M

[a] Selection line, low or high backfat depth

[b] Feed level, 0.7 or 1.2 times maintenance allowance

[c] Challenge group

APPENDIX II

Calculation of Feed Allowances

Maintenance energy requirement, for a 35kg wether lamb, was assumed to be 9.8MJME/DAY (Rattray 1986) with an additional 10% required for entire animals, making 10.8MJME/DAY. On a metabolic liveweight ($kg^{0.75}$) basis, this is 0.7MJME/$kg^{0.75}$/DAY. The chaffed lucerne hay was assumed to have an energy content of 9.5MJME/kg Dry Matter (DM) (Holmes and Wilson 1984). The proportion of DM in the feed was found to be 88.4% and therefore the maintenance allowance required per day could be derived as follows:

0.7 (MJME/$kg^{0.75}$/DAY) / (0.884 (kgDM/kg FEED) x 9.5 (MJME/kgDM))

=0.0834 kg FEED/$kg^{0.75}$/DAY for maintenance

Therefore, in order to achieve the divergent feeding levels (0.7 amd 1.2 time maintinance) used in this study, this allowance then became:

0.7 x 0.0834 = 0.0584 kg Feed/Day/$kg^{0.75}$

and

1.2 x 0.0834 = 0.1001 kg Feed/Day/$kg^{0.75}$

These figures were then used to calculate the daily allowance for each ram.

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