A STUDY OF METABOLIC DIFFERENCES BETWEEN FAT AND MEATY SOUTHDOWN SHEEP

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LIST OF ABBREVIATIONS

ADG	average daily gain
ANOVA	analysis of variance
BMR	basal metabolic rate
BSA	bovine serum albumin
BW	body weight
cAMP	cyclic adenosine monophosphate
CV	coefficient of variation
dl	decilitre
DM	drymatter
DNA	deoxyribonucleic acid
e	natural base of logarithms
g	gram
$G_{(t)}$	glucose concentration at time t
h	hour
h^2	heritability
IGF-1	insulin-like growth factor-1
IU	international unit
K_g	glucose fractional decay constant
kg	kilogram
LPL	lipoprotein lipase
mg	milligram
mm	millimeter
mM	millimolar
ml	millilitre
MANOVA	multivariate analysis of variance
MEI	metabolizable energy intake
MJ ME	megajoules metabolizable energy
MSA	mulplicative stimulating activity
NADPH	nicotinamide adenine dinucleotide
ng	nanogram
NSILA	non suppressible insulin-like factors
NEFA	non-esterified fatty acids
P	probability
r	correlation coefficient
RNA	ribonucleic acid
SED	standard error of the difference
SEM	standard error of the mean
T3	triiodothyronine
T4	thyroxine
pg	picogram
µg	microgram
µl	microlitre

Levels of Statistical Significance

NS	Not significant P>0.1
†	0.05< P <0.1
*	0.01< P <0.05
**	0.001< P <0.01
***	P<0.001

CHAPTER I

REVIEW OF LITERATURE

Introduction

The level of fatness in sheep meats is a key factor taken into account by consumers at all levels. With pricing structures offered to producers now starting to reflect this demand, there is a need for an increased understanding of the factors which affect the composition of growth so that the producer will be able to more efficiently adjust the growth patterns of young animals. The basic objective in the long term is to develop an animal which grows an increased proportion of lean tissue more efficiently (Simm and Smith 1984). Selection of lean growing animals by existing methods of body composition estimation is hampered by low correlations with true composition. The identification of metabolic markers of genetic merit for lean meat production is being attempted in the hope that these markers will improve the potential rate of genetic gain.

There is considerable natural variation in composition of growth both within and between breeds of sheep. The most variable body component is the proportion of fat (eg the fat composition of a 35 kg lamb may vary from 17-30%; Black 1983). Environmental effects explain a large proportion of this variation, but there still remains a highly significant genetic component (h^2=0.2-0.4; Wolf *et al.* 1981; Bennett *et al.* 1984; Parratt *et al.* 1987). This genetic component has been exploited in a number of within-breed selection experiments.

Selection Lines for Growth Characteristics

Selection experiments, where the selection criterion is a specific growth characteristic, provide a means of further examining the extent of within-breed genetic variation. Selection experiments also allow the physiological basis of genetic differences to be studied with the findings of such studies having two possible applications. First, the accuracy of selection for lean meat growth could be improved through the use of metabolic markers. This would be a particular advantage as existing methods of assessing fatness in the live animal are only moderately correlated with lean carcass growth (Parratt et al. 1987) and are most accurate when animals are at least 12-15 months old (Purchas et al. 1981). Metabolic markers may be more accurate predictors of the individual's genotype and/or measurable at earlier ages than other currently available methods. Secondly, an understanding of the physiological basis of gene expression may lead to the development of exogenous growth-promoting agents and, in the long term, to the identification and manipulation of specific genes controlling growth.

Many ruminant selection lines have been generated by selection on the basis of specific growth characteristics. A summary of some of the available information on these is shown in Table 1.1. Where selection has been for early liveweight (weaning weight or yearling liveweight) there has generally been a correlated increase in mature weight. This in most cases has explained a high proportion

of variation in composition on a weight-constant basis. There have been many other experiments in a range of species that have shown similar correlated responses in mature weight to selection for liveweight at earlier ages. These include studies in mice (McCarthy 1979; Brien 1987), pigs (Vangen 1980) and cattle (Newman et al. 1973; Koch et al. 1974b; Barlow 1979; Karlson 1979; Irgang et al. 1985).

Table 1.1 Response to selection for growth characteristics in ruminants.

	Breed & species	Selection criteria[a]	Realized h^{2b}	Correlated responses	Reference
1	Merino sheep	±WWT	0.25	± mature wt	Pattie et al. (1966)
2	Targhee sheep	±WWT ±WWT	0.17 0.08	+ mature & birth wt + mature & birth wt	Lasslo et al. (1985b)
3	Angus cattle	+WWT +YWT	0.30 0.38	+ mature wt + mature wt	Aaron et al. (1986b)
4	Hereford cattle	+WWT +YWT +MS	0.21 0.30 0.27	+ growth rate + growth rate + birth wt	Buchanan et al. (1982b)
5	Hereford cattle	+LGR +LCE	- -	0 growth rate + growth rate	Smith (1986)
6	Coopworth sheep	+USBF -USBF	-	0 growth rate + birth weight	Fennessey et al. (1987)
7	Southdown sheep	±USBF	-	0 growth rate	Kadim (1987)

[a] WWT=weaning weight, YWT=yearling weight, MS=muscling score, LGR=lean growth rate, LCE=lean food conversion efficiency, USBF=weight-corrected ultrasonic backfat thickness.
[b] Realized heritability of the trait selected for in the respective experiments.

Where selection criteria have included some measure of efficiency of growth or composition of growth, a correlated response in mature size has not been observed. In the case of selection for efficiency of growth this is expected as increases in mature weights

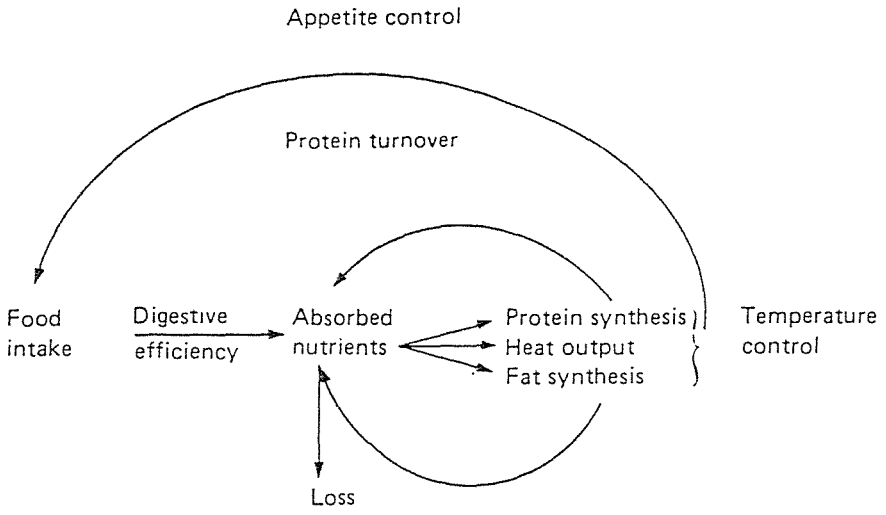
are generally associated with increased accumulated feed intakes. Hence large animals have little or no advantage in terms of efficiency (Taylor 1987). Selection for any measurement of composition on a weight-corrected basis has also tended not to result in correlated increases in growth rates or adult liveweights (Fennessy et al. 1987; Kadim 1987).

It is apparent from these ruminant experiments, including the lines of interest in this study (Experiment 7, Table 1.1), that selection for increased lean growth has not resulted in correlated increases in mature weight. This would suggest that selection on the basis of weight-corrected backfat depth has favoured strains of animals which at the same age/weight partition available nutrients differently without having associated changes in mature weight.

Variation Between Selection Lines in Nutrient Utilization

In the absence of mature weight effects, differences in composition of growth observed in the lean and fat selection lines must result from a disproportionate change in the level of one or more of the following; intake, digestive efficiency, metabolic efficiency, and nutrient partitioning (Figure 1.1).

Figure 1.1 A possible framework for the discussion of genetic aspects of growth (From Robertson 1982)



In the following sections the degree to which variation in composition of growth observed between selection lines can be attributed to variation in each of these components will be discussed.

1. Voluntary intake (appetite).

There have been many growth rate selection experiments conducted which have shown a clear positive relationship between accumulated food intake and mature body weight. These include studies in mice (McCarthy 1979; McPhee et al. 1980), pigs (Vangen 1977), cattle (Thiessen 1985), and sheep (Dodson et al. 1983; Thompson et al. 1983). In further support of the genetic relationship between appetite and mature size, cattle selected for intake have shown correlated increases in liveweight and liveweight gains (Korver et al. 1986). The key issue is, however, whether there is significant genetic variation in intake additional to that

explained by mature weight which is related to variation in the composition of growth. Selection for early liveweight (5-6 weeks) in mice appears to have exploited genetic variation in appetite (Baker et al. 1979; Roberts 1979; McCarthy 1979; McPhee et al. 1980). It appears that in the young animal food intake is the most limiting factor to growth (Robertson 1982), while in later life increased appetite in the selected line explains increased fat deposition. (Baker et al. 1979; Roberts 1979). Thompson et al. (1985) found some evidence of differences in appetite between the small and large lines of sheep above that explained by mature weight. At early ages the large line had slightly higher intake adjusted for mature liveweight. This difference appeared to be minor compared to that explained by differences in mature weight.

Selection for composition in pigs has not shown any consistent correlated increases in intake (Vangen 1980; Yen et al. 1983). Standal et al. (1985), in a review of Norwegian, French, Canadian and British pig selection experiments, concluded that there was a strong positive genetic correlation between growth rate and appetite but little or no genetic relationship between appetite and composition of growth.

In the grazing animal individual variation in diet selection is another factor which may result in an altered composition of growth. It may be that the lean genotypes select a diet which alters the intestinal flow or balance of amino acids and that this affects the net level of protein deposition. In general support of this view, the balance of amino acids, (particularly methionine, lysine,

histidine and to some extent arginine) in microbial protein is thought to limit ruminant productivity (Pisulewski et al. 1985; MacRae et al. 1986). This has been demonstrated in experiments where intestinal infusions and protection of protein from rumen fermentation have enhanced total growth and in some cases increased the proportion of lean growth (Norton et al. 1970; Orskov et al. 1976; MacRae et al. 1986; Purchas et al. 1984).

There is little information available on diet selection which applies directly to selection for composition in ruminants. However in a review of work carried out in fleece weight-selected Merinos, Clark (1987) concluded that correlated responses in diet selection were small, inconsistent and unlikely to contribute greatly to between-line variation in wool production. In an experiment carried out with rams from the Massey University Fat and Meaty selection lines (Experiment 7, Table 1.1), the meaty rams tended to select against stalk in their daily allowance of lucerne chaff (Bremmers et al. 1987). This did not result in significant between-line differences in either the nitrogen or energy digestibility of the diet consumed but the meaty line did have a significantly higher energy retention rate. A subsequent study failed to confirm this difference (van Maanen et al. 1987).

Considering the capacity of the rumen to largely buffer dietary effects of alterations in protein intake and amino acid balance (MacRae et al. 1986), and the generally low level of variability in diet selection exhibited by lines of sheep (Weston 1959; Ahmed et al. 1963), it seems unlikely that variation in diet selectivity will

be a major factor contributing to variation in the composition of growth between selection lines. However in the absence of research specifically examining selection line differences in diet selection under grazing conditions, this possibility cannot be discounted.

2. Digestive efficiency.

Digestive efficiency is a measure of the animal's ability to extract nutrients from the food that it consumes. It is generally measured simply from intake-faecal output differences (ie apparent digestibility). This provides a good estimate of the efficiency of digestion of total food intake. However, when considering specific nutrients, endogenous contributions to faecal output can greatly bias measurements.

It is generally accepted that variation between mouse selection lines in digestive efficiency is not an important source of genetic variation in gross food conversion efficiency (McCarthy 1979; Thompson 1987). In ruminants, digestive efficiency is greatly affected by many dietary factors and to some extent by the animal's physiological state and resulting appetite (Poppi 1983). However, under standardised conditions most variation in digestive efficiency is removed. Bauman et al. (1985) reviewed experiments in cows, steers, and wethers which showed that the level of variation in digestive efficiency was very low (coefficient of variation (CV) of 1.9-2.5% in animals whose productive output had a CV of 19-23%). In an experiment with fleeceweight-selected and control Romney ram lambs, McClelland et al. (1986) also reported a relatively low

level of variation in digestibility (CV =3.5%) and small inconsistent differences between the lines.

Yen et al. (1983) and Henderson et al. (1984) examined the apparent digestibility of nitrogen and energy in lean, obese and control lines of pigs. It was concluded that although the lean line retained a higher proportion of digested nitrogen, this difference was not related to digestibilities of either dietary nitrogen or energy. In an experiment with rams from the Massey University Meaty and Fat selection lines (as previously described), Bremmers et al. (1987) also reported no differences in digestibility of either nitrogen or energy.

3. Metabolic efficiency.

Metabolic efficiency of a sheep is defined as the proportion of digested energy which is deposited in or secreted as tissue, milk, conceptus and wool. In theory the difference between that energy digested and that secreted or deposited will be lost as heat and/or dispatched as some form of work. In the following section, factors which have been shown to explain variation in heat production, and the extent to which these can be related to between-line variation in composition of growth, will be discussed.

Components of heat production have been further identified as; basal or fasting metabolism (BMR), heat of activity, heat of digestion, absorption and assimilation, heat of thermoregulation, heat of fermentation and heat of waste formation and excretion. Of

the above components, basal heat production is the major contributor to total heat production and is likely to be the dominant source of variation in heat production (Baldwin et al. 1984). Basal heat production has been shown to be an approximate function of surface area (ie metabolic body weight, $BW^{2/3}$). The underlying concept is that the energy cost of maintaining body temperature is a function of the surface area to volume ratio. Examination of a range of species has shown that BMR is best described by the equation $y = a\ BW^{0.75}$. The adoption of 0.75 as the exponent has been largely on empirical grounds, estimates having been shown to vary from 0.4-0.85 (Baldwin et al. 1984). The value of the coefficient "a" accounts for variable effects of activity, thermoregulation, and physiological state. Estimates of these coefficients are readily available from feeding tables generated from experimental data.

There is little information available which attributes basal heat production to specific physiological or metabolic processes. In general, functions which contribute to basal heat production are ; (a) service functions such as those of the heart, liver, lungs, kidney and accompanying endocrine and nervous integration and control, and (b) tissue cellular level functions such as maintenance of membrane potentials and macromolecule resynthesis (Baldwin et al. 1984).

Considerable variation in BMR observed between lambs and adult ewes, and between cold-stressed and unstressed lambs, has been attributed to ATP-dependent Na^+ and K^+ transport (Baldwin et al.

1984). It appears that thyroid hormones may regulate this heat production by affecting the level of Na^+ and K^+ pumping. Another source of variation which has explained effects of both breed and physiological state on BMR is relative organ weights (Thompson 1987). Baldwin et al. (1984) reviewed experiments in lactating vs. non lactating dairy cows and rats and in high- vs low-fed pigs and sheep. All studies showed strong positive correlations between BMR and relative weights of the visceral organs and gastrointestinal tract. The energy expenditure of these tissues appears to account for a large proportion of total maintenance requirements and is related to high rates of protein synthesis in these organs. In a review of experiments with lactating cows, genetic merit for milk production was also shown to be positively correlated with visceral organ and intestine weights and maintenance requirements. Furthermore, the higher relative maintenance costs of high producing cows in a good environment are thought to explain the genotype by environmental interaction observed when these cows are apparently at a disadvantage in a restrictive environment (Ferrell et al. 1985). In a contrasting review Bauman et al. (1985) concluded that cows in the same physiological state consuming the same diets showed a low level of variation in maintenance requirement while exhibiting a much greater degree of variation in productive output.

In an experiment with growing sheep Leymaster et al. (1985) showed a moderate correlation between rate of offal protein accretion and growth rate ($r=0.58$). Low but significant correlations between offal protein accretion rate and compositional

traits were also shown (eg r=-0.33 between offal protein accretion rate and carcass fat composition). It was suggested on this basis that accretion rate of offal has effects on partitioning of nutrients between heat production, protein and fat.

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 organs in the fat line were thought to be the explanation for this difference. In a later experiment (Yen et al. 1985), with animals drawn from the same selection lines, lower levels of circulating triiodothyronine (T3) were found in the fat line, although thyroxine (T4) levels were not different. T3 is thought to be the active form of the thyroid hormones in terms of effects on heat production, and therefore may also be related to the differences in BMR between these lines. York et al. (1978) showed similar differences in T3 concentrations between obese and lean mice.

The largest and most consistently reported effect on BMR appears to be the relative weights of the visceral organs and intestines. It appears that the rapid rate of protein synthesis in these tissues is the explanation for this effect. Thus the correlation between the rate of protein synthesis and fasting heat production has been shown to be 0.7-0.95 and protein synthesis in the visceral organs and intestines accounts for approximately 50% of total body protein synthesis (Yen et al. 1983; MacRae et al. 1986).

In three experiments with rams drawn from the Massey University Fat and Meaty selection lines (Experiment 7, Table 1.1) the Meaty line invariably exhibited slightly higher organ weights (Kadim 1987). However the differences did not consistently reach statistical significance. Considering this observation, the relatively small differences in carcass protein content (approximately 5%; Kadim 1987) and the relatively slow rate of protein synthesis in skeletal muscle (MacRae et al. 1986), it seems unlikely BMR will greatly differ between the Fat and Meaty selection lines of interest in this study.

4. Nutrient partitioning

Much variation in the relative rates of fat and protein deposition observed between breeds and different strains of the same breed can be accounted for by mature size effects. In general an animal with a potentially larger mature size, when compared at the same weight, will be relatively less mature, growing faster, and be depositing less fat (Webster 1977). However, in this discussion variation in nutrient partitioning above that explained by mature size effects is of primary interest. It appears from selection experiments carried out in a range of species that there is considerable genetic variation in nutrient partitioning and that this is likely to be the dominant source of genetic variation in the composition of growth, particularly considering the generally low level of variation in digestive and metabolic efficiency. Mice selected for weight gain under a restricted feeding regime are thought to have exploited genetic variation in nutrient

partitioning. Because the energy cost of fat deposition is considerably higher than that of wet protein deposition (30 vs. 5 MJ/kg, Webster 1977; 5:1 ratio, Baldwin et al. 1984), selection for growth rate has tended to favour lean growth (Hetzel et al. 1978). Further illustration of the relative energy costs of fat vs protein deposition has been shown in a comparison of tissue deposition efficiency in Charolais and Friesian cattle. Higher efficiency of tissue deposition in Friesians was related to increased partitioning of nutrients into protein in this breed (Geay et al. 1979). Similarly bulls fed ad-lib, which tend to deposit more protein than steers, have higher basal heat production associated with the higher energy cost of protein deposition. However, they are more efficient than steers because of the lower relative energy cost of wet protein deposition (Baldwin et al. 1984). Tess et al. (1984) reviewed a number of experiments in sheep, pigs and rats and although in most cases there was an energetic advantage in protein vs fat deposition, this was not always the case. Furthermore in an experiment with lean and fat selection lines of pigs Tess et al. (1984) concluded that genetic variation in energetic efficiency may exist independently of any association with lean mass and total protein and fat deposition.

Where ruminants have been selected for lean growth there has tended not to be any correlated increase in mature size (as previously discussed). It also seems unlikely that the responses to selection have resulted from correlated responses in intake, digestive or metabolic efficiency. In this light the Meaty and Fat lines of interest in this study seem most likely to differ in the

way they control nutrient partitioning between fat and protein deposition. In the following sections neuroendocrine factors which are known to be involved in the control of protein and fat deposition will be discussed.

Endocrine Control of Nutrient Deposition

1. Protein

The rate of skeletal muscle protein deposition at any particular age is well explained by the animal's relative maturity. It is now generally accepted that, in the absence of nutritional restrictions and/or imbalances of permissive factors (such as insulin and thyroid hormones), growth hormone and related somatomedins initiate and control this skeletal growth of bone and protein (Goldberg et al. 1980). However factors which invoke increased lean growth above that explained by mature size effects are of particular interest in this study.

A. Factors affecting myogenic cell proliferation in prenatal and early life

There is considerable evidence which suggests that potential muscle mass is determined prenatally, or in early in life, by the level of muscle DNA replication. Muscle DNA accumulates post-natally as a result of muscle satellite cell replication which serves to add new DNA to existing muscle fibres. One hypothesis is that the level of DNA replication is related to the number of precursor satellite

cells which are present at or close to birth (Allen et al. 1979). The degree to which satellite cells contribute to myofibril DNA content is thought to affect the potential level of cellular protein accumulation. In support of this Hoffman et al. (1983) and Purchas et al. (1985) reported lower numbers of satellite cells showing proliferative activity in fat selection lines of pigs and mice respectively. Lord et al. (1986) also reported an increased in vitro incorporation of labelled thymidine into muscle of young lambs from a lean selection line (as previously described, Experiment 6, Table 1.1) compared to that of contemporary lambs from the fat line. The rate of muscle thymidine uptake is thought to be related to the rate of DNA synthesis.

There is little known about the endocrine factors which influence the replication of the myogenic precursor cells prenatally. Work reviewed by Hoffman et al. (1983) seemed to indicate that hypothalamic factors are not involved to a large extent as it has been shown that decapitated foetal pigs continue to grow normally to birth. However in one experiment, Hoffman et al. (1983) were able to relate differences in foetal composition of genetically lean and obese pigs to differences in plasma growth hormone levels. In further support of hypothalamic factors having control over early muscle cell replication, Hausman et al. (1987) showed that hypophysectomized pig foetuses had reduced activities of muscle fibre ATPase and other enzymes indicating delayed fibre type differentiation.

There has also been much speculation as to the involvement of placental factors such as prolactin and placental lactogen which may

be related to foetal liver production of insulin-like growth factors (IGF's) (Allen et al. 1979). Studies with cultured foetal muscle tissue have shown clear effects of IGF-1 on the rate of myogenic cell proliferation. (Atkinson et al. 1987).

Postnatal treatment of young animals with exogenous growth hormone has shown clear increases in both growth rate and the relative rate of protein deposition (Chung et al. 1985; Pell et al. 1987). Plasma concentrations of growth hormone have also been shown to be higher in pigs from lean selection lines (Ringberg Lund-Larsen et al. 1975; Althen et al. 1976). Kotts et al. (1987) have conducted an experiment which suggests that the effects of growth hormone on growth are partly attributable to increased promotion of myogenic cell proliferation. This experiment showed that sera from young pigs treated with growth hormone caused generally higher levels of in vitro myogenic cell proliferation than pre-injection sera. IGF-1 levels were only moderately correlated with the magnitude of the proliferative response. This suggests that there are additional factors elevated by growth hormone which have not been identified. Other peptide factors which may be involved in the stimulation of muscle cell replication include multiplicative stimulating activity (MSA), nonsuppressible insulin-like factors (NSILA), and fibroblast growth factor (reviewed by Allen et al. 1979).

B. Postnatal endocrine effects on muscle cell hypertrophy

Skeletal protein is a very dynamic tissue, net protein deposition being a function of the difference between simultaneous synthesis and degradation. During growth the rate of degradation is almost as rapid as the rate of synthesis. eg in a growing pig 2 grams of protein synthesised is matched by 1 gram degraded (Waterlow 1984). In growth both the rate of synthesis and the rate of degradation are elevated. For example, in young mice the rate of protein degradation is 3-7 times that of adult mice (Waterlow 1984). It seems that mechanisms which control the balance of protein synthesis and degradation will be major determinants of the animal's surplus energy levels as the energy expenditure in protein synthesis is a relatively high proportion of the total energy expenditure (eg 15% of MEI in a lamb growing at 200g/d; Waghorn et al. 1984).

Increased synthesis results from increased translational capacity of the muscle cells (the number and capacity of the ribosomes and in some cases the amount of mRNA) and degradation by increased activity of proteinases and the lysosomal system. Control of the relative activities of these mechanisms is not fully understood. There may be intracellular links and/or outside influences which coordinate these processes. Insulin, growth hormone, thyroid hormone and glucorticorticoids have all been shown to have effects on the relative rates of protein synthesis and degradation (Goldberg et al. 1980).

i. Insulin

Insulin is thought to partly explain the decreased protein degradation and enhanced protein synthesis which occur in response to feeding. Increased insulin concentration has been shown to increase muscle cell amino acid uptake and incorporation into protein *in vitro*. However, the presence of amino acids and glucose alone have also been shown to have this effect (Goldberg *et al.* 1980). In support of insulin having a role in controlling protein metabolism, exogenous insulin treatment of alloxan diabetic cattle has been shown to restore plasma urea to pretreatment levels and to stimulate cellular uptake of branched-chain amino acids (Prior *et al.* 1983). Sumner *et al.* (1983) also showed that sheep infused with insulin had reduced plasma urea levels and urea excretion rates. Assuming equal pool sizes and plasma urea clearance rates, differences in plasma urea concentrations are likely to reflect differences in the rate of protein degradation (Goldberg *et al.* 1980).

Insulin is intimately involved in the control of amino acid uptake and the supply of energy to the muscle cell, and is a necessary co-factor to growth hormone for liver synthesis of IGF's (Allen *et al.* 1979). However circulating levels of insulin have been more often negatively associated with protein deposition (Trenkle *et al.* 1978), and are therefore not thought to be related to between-line differences in protein deposition.

ii. Growth hormone

Goldberg et al. (1980) reported that hypophysectomised rats had reduced rates of protein synthesis, protein degradation and protein gain. Growth hormone infusions restored the rate of protein synthesis but only in the presence of low levels of thyroid hormone. Exogenous treatment of lambs with growth hormone (Chung et al. 1985; Butler-Hogg et al. 1987; Pell et al. 1987) and of steers with growth hormone-releasing factor (Moseley et al. 1987) have shown clear increases in the net rate of protein deposition. These animals showed a corresponding increase in nitrogen retention rates, apparently in the absence of effects on digestive efficiency. Selection for lean growth in pigs has shown correlated responses in basal levels of growth hormone and IGF's (Althen et al. 1976, Ringberg Lund-Larsen et al. 1977).

There seems to be little doubt that growth hormone has effects on the net rate of protein deposition. It also seems likely that the effects of growth hormone are invoked both via the related IGF's (Ringberg Lund-Larsen et al. 1975) and by direct effects on muscle cells (Florini 1985). The information available does not allow conclusions to be drawn as to whether the observed increase in protein deposition rates results from relative increases in synthesis, decreased rates of protein degradation, or both. However, somatomedins (IGF I&II) and growth hormone have been shown to increase muscle cell substrate uptake, ribosome activity,DNA synthesis and protein synthesis in vitro (Florini 1985; Froesch et al. 1985).

iii. Thyroid hormones

Exogenous thyroid hormone treatment has been shown to restore protein degradation rates in hypophysectomised rats to levels equal to that of a control group. Furthermore, it is thought that the wasting of muscle in cases of hyperthyroid activity is related to thyroid-stimulated degradation. Thyroid hormone is also considered a permissive factor to protein synthesis (Goldberg et al. 1980; Florini 1985).

There appears to be some interaction between thyroxine and the effects of growth hormone and related 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 growth hormone releasing factor in stimulating hypothalamic production of growth hormone (Leung et al. 1985) and also be a necessary co-factor for growth hormone-stimulated synthesis of IGF's (Froesch et al. 1985). The significance of these interactions in terms of the composition of growth remains unknown. However, thyroid hormone differences have been shown between selection lines of pigs and mice, as previously discussed, and these were associated with differences in heat production. It is possible that the differences in heat production could be related to higher relative rates of protein synthesis in the lean lines.

iv. Glucocorticoids

Glucocorticoid treatment of rats was associated with decreased rates of protein synthesis and amino acid uptake and increased rates of protein degradation (Goldberg et al. 1980; Florini 1985). Treatment of domestic species with high doses of glucocorticoids has been shown to decrease growth rate, and is believed to have direct suppressive effects on muscle cell DNA and protein synthesis while also increasing the fractional protein degradation rate (Spencer 1985). Glucocorticoids at physiological levels seem to be principally involved with the mobilisation of amino acids and energy substrates while the animal is in negative energy balance (Spencer 1985). However there is some evidence that glucocorticoids given in low doses stimulate proliferation of primary rat myoblasts and glucose uptake (Florini 1985). Some in vivo studies have also shown increased growth rate and carcass fat content in lambs treated with cortisol analogues, although results have been variable (Purchas 1972). Adrenalectomy has also been shown to abolish the increased lipid and decreased protein content of growth observed in obese Zucker rats (Fletcher 1986).

2. Carbohydrate and fat

The principal role of carbohydrate and lipid metabolism is to maintain energy homeostasis. Thus differences in fat deposition may be secondary to changes in other aspects of metabolism which have affected the level of surplus energy. The alternative is that changes in carbohydrate or fat metabolism results in changes in the

animal's gross efficiency, and therefore affect the amount of surplus energy.

It is generally accepted that obesity arises irrespective of the genetically-determined number of adipocytes. Increased fatness observed in fat selection lines of pigs (Hood 1983) and sheep (Kadim 1987) has largely been a consequence of increased fat cell hypertrophy . Some studies have shown increases in the number of subcutaneous adipocytes in fattening. However it appears that these increases in fat cell number are a result not of cell replication but of recruitment of pre-adipocyctes which are only identifiable after fat is deposited in them. Attempts to prevent fat deposition by reducing the number of adipocytes in early life have been unsuccessful (Wood 1982).

A. Insulin

In humans, obesity has often been associated with elevated basal insulin levels and exaggerated response of plasma insulin to exogenous glucose infusions. These differences are thought to be a result of relative tissue insensitivity to insulin and/or increased pancreatic sensitivity to glucose (Waghorn et al. 1984). Similar differences have been shown between lean and fat selection lines of pigs (Wangsness et al. 1977; Mersmann et al. 1982), fat milk-fed lambs and lean early weaned lambs (Munro et al. 1984), lean and fat adult sheep (McNiven 1984), and lean and obese heifers (McCann et al. 1985a,b). McCann et al. (1985b) suggested that, in most cases, the relative insulin insensitivity was a result of decreased numbers of insulin receptors.

It is thought that the generally higher level of circulating insulin in fat animals provides greater suppression of lipolytic stimulus and that this tends to increase the animals' efficiency. In support of this view, insulin has been shown to reduce the lipolytic effect of adrenalin in cattle (Jones et al. 1983). Pigs from fat selection lines with higher basal insulin levels showed lower lipolytic responses to exogenous adrenalin (Standal et al. 1973,1979; Mersmann et al. 1982). Brocklen et al. (1986) also showed reduced lipolytic responses of fat Pietrain pigs injected with adrenalin. It appeared that the reduced response was related to reduced numbers of adrenergic receptors. This effect could be independent of the effects of higher insulin levels. It appears from this information that reduced lipolytic sensitivity of pigs from fat selection lines may contribute to their decreased tendency to deposit lean tissue.

Another possible effect of insulin is that it is associated with the animal's intake control mechanism. It has been demonstrated in human-related research that insulin binds hypothalamic receptors which provides stimulus to the central nervous system and possibly affects the satiety centre (Posner 1987). However, in ruminant experiments, exogenous insulin has not been demonstrated to affect intake (Baile et al. 1974). There is evidence that insulin can act via the hypothalamus to cause peripheral uptake of glucose and affect hepatic glycogen metabolism independent of direct effects of insulin in humans (Posner 1987). However the presence or importance of such a stimulus in ruminants is largely unknown.

Lord (1986) was not able to show any differences in the glucose-insulin status of sheep drawn from lean and fat selection lines (Experiment 6, Table 1.1) and the importance of insulin in controlling rates of lipogenesis in ruminants is still in question (Thornton et al 1982).

B. Sympathetic nervous system

The sympathetic nervous system has also been shown to have effects on the rate of lipolysis. Electrical stimulus increases free fatty acid mobilisation, and denervated tissue exhibits increased lipid mass, while pharmacological abolition of sympathetic activity inhibits normal mobilisation of lipids (Trayhurn et al. 1987). It is thought that sympathetic control of lipolysis is important in short term mobilisation of lipid, but of little importance in the long-term control of lipid metabolism (Wood 1982).

C. Specific lipogenic enzymes

Differences in fatness between genotypes may arise from differences in lipogenic rates as well as in lipolytic rates. Lipoprotein lipase is responsible for binding of lipoproteins at the capillary interface for fatty acid uptake. Some research has indicated that LPL activity is higher in fat than in lean selection lines of pigs (McNamara et al. 1982). A similar difference was demonstrated in foetal pigs, but it had largely disappeared soon after birth (McNamara et al. 1982). This may indicate that the level

of LPL has a direct bearing on the level of adipose tissue deposition. However against this the effects are not shown later in life when a difference would potentially have the greatest effect on fat deposition. The synthesis of fatty acids by the de novo pathway is generally limited by the supply of reducing equivalents (NADPH). In pigs 50-80% of NADPH required for fatty acid synthesis is produced in the initial steps of the pentose phosphate pathway catalyzed by the enzymes glucose-6-phosphate dehyrogenase (G-6-PD) and 6-phosphoglucanate dehydrogenase (6-PGD). The remainder is produced by the oxidation of citrate to pyruvate in a pathway catalysed by enzymes malic dehydrogenase, the citrate cleavage enzyme, and NADP iso-citrate dehydrogenase. Many of these enzymes have been shown to be elevated in fat lines of pigs (Steele et al. 1972; McNamara et al. 1982; Rogdakis 1982; Rothfuss et al. 1984) and rats (Hood 1983) Furthermore the activity of these enzymes has been shown to be highly heritable in pigs (h^2 =0.4-0.7) and to have a genetic correlation with carcass fatness of 0.4-0.7 (Strutz et al. 1977; Standal et al. 1979).

Physiological Characteristics as Potential Predictors of Genetic Merit for Lean Meat Production.

The identification of markers, such as blood metabolites or hormone concentrations, which are associated with genetic merit for lean meat production could greatly benefit the meat industry. Measurement of physical characteristics, such as backfat thickness (the most common method of estimating the animal's relative fatness), are poorly correlated with total body composition which in turn is only moderately correlated with genetic merit because of large environmental influences. Moreover backfat thickness cannot be accurately measured early in life.

To be an effective alternative to existing methods of selection, physiological markers should meet the following criteria : 1) they should be more highly correlated with genetic merit for lean meat growth than existing methods and/or be measurable at earlier ages, such that the potential rate of genetic gain is increased. 2) they should be easily and cheaply measured such that the cost incurred in their measurement does not exceed the value of any long term genetic gains. This would require not only low cost assays, but also that the conditions required for sampling be easily achieved. 3). they should have no significant negative correlations with other economic traits. Although, as discussed earlier, numerous physiological differences have been associated with genetic merit for lean growth, physiological characteristics which meet all these criteria as markers for lean meat production in ruminants have yet to be identified.

CHAPTER II

EXPERIMENTAL

PLASMA METABOLITE AND HORMONE CONCENTRATIONS AS PREDICTORS OF GENETIC MERIT FOR LEAN MEAT PRODUCTION IN SHEEP: EFFECTS OF METABOLIC CHALLENGES AND FASTING

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Abstract

Genetic variation in the potential of domestic animals to grow lean vs fat tissue may be related to homeorhetic control of metabolism. To examine this hypothesis, a comparison was made of the responsiveness of 10 rams from each of the Massey University Fat and Meaty Southdown selection lines to exogenous metabolic challenges. Challenges involved acute intravenous doses of adrenalin, glucagon, insulin and glucose and a 64h fasting period.

Relative to the Fat line, the Meaty rams exhibited: (a) lower baseline concentrations of plasma urea during both the fasting and refeeding periods; (b) higher growth hormone concentrations over the fasting and refeeding periods; (c) increased lipolytic responses to a glucagon challenge; and (d) larger glucose distribution spaces. No significant between-line differences were detected in responsiveness

to the adrenalin or insulin challenges. It is concluded that baseline plasma urea concentrations, and responsiveness to glucagon and glucose challenges may provide useful predictors of genetic merit for lean meat production. However it is not known whether these differences would be exhibited under less standardised conditions and hence have application under field conditions.

Introduction

Selection experiments have shown that composition of growth is a heritable trait, and that selection on the basis of weight-corrected backfat measurements can achieve genetic improvement of meat-producing animals (Beatson 1987). However, the measurement of backfat thickness in sheep can only be made with reasonable accuracy when considerable fat has been deposited, and backfat depth tends to be only moderately correlated with whole animal composition. Response to selection is likely to be reduced because of the errors associated with backfat measurement and because measurements are best made later in life (which results in an increased generation interval). Physiological markers of genetic merit for lean meat growth could reduce errors of prediction, so potentially allowing earlier selection of breeding stock and improving the rate of genetic gain.

Physiological differences between fat and lean animals which might provide possible markers of genetic merit for lean growth have been identified in a number of studies. Plasma concentrations of growth hormone have been positively related to liveweight gain and

negatively related to fatness in cattle (Trenkle et al. 1978). Althen et al. (1976) and Wangsness et al. (1977) showed that pigs selected for fatness had lower serum growth hormone concentrations than the unselected controls or lean selection lines. Ringberg Lund-Larsen et al. (1975) showed that pigs selected for low backfat and high growth rate had higher serum concentrations of both growth hormone and somatomedin-C (insulin-like growth factor-1).

Plasma urea concentration have also been observed to differ between a number of selection lines. These include : dairy cows selected for high vs low milk yield (Sejrsen et al. 1984, Sinnet-Smith et al. 1987); Romney sheep selected for high fleece weight vs controls (Clark et al. 1987, McCutcheon et al. 1987) and pigs selected for low vs high fatness (Mersmann et al. 1984). In each case the less productive line maintained higher concentrations of plasma urea.

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 had higher basal concentrations of glucose and/or insulin, reduced peripheral sensitivity to insulin and, in some cases, increased pancreatic sensitivity to glucose. Exogenous adrenalin 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).

Such differences between genotypes indicate that basal concentrations of some hormones and metabolites may be genetically

correlated with the composition of growth. Differences in tissue sensitivity/responsiveness to homeostatic factors may also indicate between-selection line differences in homeorhetic control of metabolism (Bauman et al. 1980). These differences, if repeatable, might therefore be used as physiological markers of genetic merit. The objective of this study was to examine the possibility that differences in baseline metabolite/hormone concentrations or sensitivity to metabolites/hormones exist between the Massey University Fat and Meaty selection lines of Southdown sheep.

Materials and Methods

1. Animals

Ten seven-month old ram lambs were selected from each of the Massey University Meaty and Fat selection lines of Southdown sheep. The divergent selection experiment from which the rams were drawn was initiated in 1976 with approximately 100 commercial Southdown ewes allocated to either the Meaty or Fat lines on the basis of weight-corrected backfat measurements (Purchas et al. 1981; Purchas et al. 1982). Fat depths were measured using an ultrasound backfat probe as described by Gooden et al. (1980). Subsequent selection of breeding stock and culling of mixed age ewes was also based on weight-corrected backfat measurements. Each line has been maintained at approximately 70 ewes which are first mated at 18 months of age. Two 18 month-old sires are used annually in each line. Approximately 3.0 generations had elapsed from the time that the selection program commenced to the time that lambs used in this experiment were born.

Selection of the experimental animals was at random within each of the sire groups and was balanced with regard to birth and rearing status of the lambs. The rams were housed in metabolism crates and fed a daily ration of chaffed lucerne hay to approximately 30% above maintenance. Maintenance requirement was calculated as 0.7 MJ ME/$kg^{0.75}$ and the feed was assumed to contain 10 MJ ME/kg DM. A mineral supplement (59% sodium chloride, 37% sodium sulphate, 4% sodium molybdate) was added to the ration at a rate of 1 g/day to avoid possible copper toxicity problems. Feed was offered once daily at 1630 h and fresh water was available ad libitum.

2. Experimental Procedure

Following a 27-day adjustment period, the mean responses of the lines to four metabolic challenges and a 63 h fasting period were compared. Rams were randomly allocated into five blocks (balanced on sire), and individuals in each block were treated simultaneously. The metabolic challenges and their respective injection times, concentrations and dose rates are presented in Table 2.1.

Table 2.1 Metabolic challenges, injection concentrations and dose rates administered to 10 Fat and 10 Meaty Southdown ram lambs

Time of administration Day	Time (h)	Metabolite/ hormone	Dose rate (/kg)	Injection concentration (/ml)
I	0900	Adrenalin[a]	1.0µg	7 µg
	1300	Insulin[b]	0.01mg	0.07mg
II	0900	Glucagon[c]	0.175µg	2 µg[d]
	1300	Glucose	0.17g	0.5g[e]
Fasting period commenced following completion of feeding Day I.				
IV	0830	End of fast (Fed half daily ration)		

[a] Lot 78K021, McGaw Ethicals Ltd, Auckland, New Zealand.
[b] I-5500 Lot 1216-1350, Sigma Chemical Co., St. Louis, Mo., USA.
[c] G-4250, Sigma Chemical Co., St. Louis, Mo., USA.
[d] Dissolved in 3% bovine serum albumin (Lot 103c-0446, Sigma)
[e] McGaw Ethicals Ltd, Auckland, New Zealand.

To facilitate injections and blood sampling, jugular vein cannulae were inserted on the day before the first challenge. The cannulation site was clipped and washed with 70% ethanol then local anaesthetic (10% Xylocaine; Astra Pharmaceuticals Ltd.,N.S.W., Australia) applied as a surface spray. After 2-3 minutes an Angiocath intravenous placement unit (12G needle and sleeve; Sherwood Medical, St. Louis Mo., U.S.A.) was inserted through the skin and into the jugular vein. The needle was withdrawn and a sterile cannula (Internal diameter 1mm, external diameter 1.5mm, Dural Plastics and Engineering, N.S.W., Australia) was passed through the sleeve and 100mm into the vein. The sleeve was then withdrawn and the cannula fixed in place by wrapping at the wound site with plastic tape (Sleek Tape, Smith and Nephew Med. Ltd., Hull, England), embedding suture material in the tape and passing a

single suture through the skin. The cannulae was then passed to a point behind the head and held in place with 75mm elastic adhesive bandage. Antibiotic (Streptopen, Lot 312620, Glaxo, N.Z.) was administered (5ml) and rectal temperatures were taken daily. No signs of elevated rectal temperatures were observed during the experiment. Patency of cannulae was maintained with 0.9% saline containing 100IU/ml heparin (Batch 128B, Wendal Pharmaceuticals Ltd, London).

With the exception of glucagon, the challenges were dissolved in saline (0.9% sodium chloride) immediately prior to injection. Glucagon was dissolved in saline containing 3% bovine serum albumin (BSA), to a concentration of 4ug/ml and frozen at -12°C until immediately before the challenge. It was then thawed and further diluted to the injection concentration of 2ug/ml.

Blood sampling associated with each challenge was carried out in the following time sequence : -30, -15, -5, -2, 2.5, 5, 7.5, 10, 15, 20, 30, 45, 60, 75, 90 and 120 minutes relative to the time of challenge. Challenges were administered at time zero and were followed by 6 ml of saline to clear the catheters. Samples were also obtained at 0, 24, 39.5, 51.5, 63.5 and 64 hours following the feeding on day I and subsequent to refeeding on day IV after 0.5, 1.0, 1.5, 3.5 and 7.5 hours. Approximately 6 ml of blood was removed at each sampling time, placed in chilled tubes (containing sodium citrate as the anticoagulant), and immediately centrifuged. Plasma was harvested and frozen in duplicate tubes at -12°C until subsequent analysis.

3. Determination of Plasma Hormone and Metabolite Concentrations

Concentrations of glucose, non-esterified fatty acids (NEFA) and insulin were measured to evaluate responses to the four metabolic challenges. Plasma concentrations of glucose, β-hydroxybutyrate, insulin, NEFA, urea and growth hormone were measured over the fasting and refeeding periods. Pooled baseline samples were taken from each of the pre-challenge samples for determination of growth hormone and Insulin-Like Growth Factor-1 (IGF-1) concentrations.

Glucose concentrations were determined using a YSI Model 27 industrial analyzer (Yellow Springs Instrument Co., Colorado). β-hydroxybutyrate was analysed enzymatically using β-hydroxybutyrate dehydrogenase (Sigma Chemical Co.) following the method of Williamson and Mellanby (1974). Plasma concentrations of NEFA were determined by the modified colourimetric method described by McCutcheon et al. (1986) and growth hormone and insulin concentrations using the heterologous double antibody radioimmunoassay systems described by Flux et al. (1984). Plasma urea concentrations were measured using the autoanalyser method of Marsh et al. (1965). Further details of these assays are provided in the appendix. Concentrations of IGF-1 were measured by a radioimmunoassay procedure following acid-ethanol extraction as described by Gluckman et al. (1983).

4. Glucose Tolerance Test

The characteristics of individual animal responses to the glucose challenge were analysed on the basis of the relative disappearance constants (K_g) and the theoretical time zero glucose concentrations ($G_{(0)}$). It was assumed that the glucose load was instantaneously distributed into one theoretical compartment (ie the model $G_{(t)} = G_{(0)}e^{(-txKg)}$). This model was fitted to the glucose response data in the period from 5 to 30 minutes following the glucose injection. Glucose distribution space was calculated from $G_{(0)}$ and the glucose dose. A parameter of insulin resistance was calculated from the insulin response to the glucose challenge by the method described by Wastney (1982). This parameter is the product of the glucose half life (t1/2) and the average extra-vascular insulin concentration, also in the period 5-30 minutes after injection. Extra-vascular insulin concentrations were predicted using the human model of insulin kinetics described by Sherwin et al. (1974). This is a three compartment model, compartment 1 is the plasma space. The other two compartments are extravascular; compartment 2 is small and equilibrates rapidly with plasma, and compartment 3 is large and equilibrates slowly with plasma.

5. Statistical Analysis

Effects of block, selection line and sire-within-selection line on metabolite/hormone concentrations (Model 2.1) were compared at each sampling time by analysis of variance (ANOVA) using the generalized linear model computer package 'REG' (Gilmour 1985).

Post-challenge hormone/metabolite concentrations were corrected for pre-challenge concentrations (by subtraction of the mean pre-challenge concentrations). Baseline-corrected data were then subjected to ANOVA to test between-line differences. Where the effect of block and/or sire was non-significant the appropriate reduced model was used. In cases where significant between-line differences were detected at consecutive sampling times, multivariate analysis of variance (MANOVA) was used to provide a pooled level of statistical significance of line effects over that interval.

Model 2.1 $y_{ijk} = \mu + \alpha_i + \beta_j + \Theta_{jk} + e_{ijk}$

y_{ijk} = ijkth metabolite/hormone concentration
μ = arithemetic mean
α_i = effect of ith block (i=1...5)
β_j = effect of jth selection line (j=1...2)
Θ_{jk} = effect of kth sire within jth selection line
e_{ijk} = ijkth residual

Homogeneity of the within-selection line regressions of blood metabolite concentrations on backfat thickness was tested by the method of Searle (1971).

Results

1. Liveweights, liveweight gains and backfat measurements

The mean fasted liveweight of the lambs was 30.7 ± 0.7 kg (Mean ± SEM) at the beginning of the experimental period. There were no significant effects of line or sire within line on birth weight, liveweight gain from birth to the start of the experiment, or liveweight gain during the experimental period. Subcutaneous backfat

measurements taken two months after completion of the experiment are presented in Table 2.2. The fat line group had significantly greater backfat depths than the meaty line while the effect of sire within line was non-significant.

Table 2.2 Animal liveweights, liveweight gains and backfat measurements of 10 Fat and 10 Meaty Southdown ram lambs

	Selection Line			SED[a]
	Fat	Meaty		
Birth weight (kg)	3.6	3.7		0.1
Fasted weight[b] (kg)	30.6	30.7		0.7
ADG[c] from birth (g/day)	154.5	151.9		3.8
ADG[c] exp[d] (g/day)	70.8	55.0		7.5
Backfat depth (mm)	3.9	2.3	***	0.2

[a] Standard error of the difference
[b] at the beginning of the experiment
[c] average daily gain
[d] ADG over the experimental period

2. Baseline metabolite/hormone concentrations

Mean plasma concentrations of glucose, insulin and NEFA for the four pre-challenge samples from each of the metabolite/hormone challenges are presented in Table 2.3. IGF-1, growth hormone and urea concentrations (determined from the pre-challenge samples which were bulked for analysis) are also presented in Table 2.3.

Table 2.3 Baseline plasma concentrations of growth hormone, IGF-1, urea, glucose, insulin and NEFA in 10 Fat and 10 Meaty Southdown ram lambs

Metabolite/hormone	Selection Line		SED[a]
	Fat	Meaty	
GH (ng/ml)	2.0	2.8	0.4
IGF-1 (ng/ml)	30.0	32.7	1.2
Urea (mM)	8.9	7.9 ***	0.1
Glucose (mg/dl)	75.2	74.6	1.1
Insulin (pg/ml)	1382	971	245
NEFA (ueq/l)	105.6	114.6	5.2

[a] Standard error of the difference

Fat animals exhibited significantly greater baseline concentrations of urea than the meaty animals but all other differences were non-significant.

Relationships between baseline concentrations of these metabolites/hormones, liveweight gain from birth and weight-corrected backfat depth were also examined. Significant ($P<0.05$) positive correlations were evident between: liveweight gain from birth and IGF-1 ($r=0.57$); plasma urea and weight-corrected backfat depth ($r=0.58$); and insulin concentration and weight-corrected backfat depth ($r=0.47$).

Within-line regression coefficients of liveweight gain on IGF-1 concentration were not significantly heterogeneous ($P>0.1$) and the pooled regression was significantly different from zero ($P<0.05$). Conversely, the significant correlation between plasma urea concentration and weight-corrected backfat depth was largely a result of the significant between-line variation in blood urea concentrations and backfats. Intra-line regressions of weight-

corrected backfat depth on urea and insulin concentrations were not significantly different from zero. Examination of raw data revealed that the significant correlation between insulin concentration and weight-corrected backfat depth was largely attributable to one individual ram with exceptionally high values for both parameters. With data from this animal removed the correlation became non-significant ($P>0.1$). Correlations between growth hormone, IGF-1, glucose, NEFAs, and weight-corrected backfat depth were low and non-significant.

3. Adrenalin Challenge

Plasma concentrations of glucose and NEFA in response to the 0.1 µg/kg adrenalin challenge are presented in Figure 2.1. Glucose concentration rose to peak at approximately 5 minutes after the injection while plasma NEFA concentrations peaked at approximately 7.5 minutes following the injection. No significant between-line or sire-within-line differences were detected at any time in either of the metabolites, even when correction for pre-challenge concentrations was made.

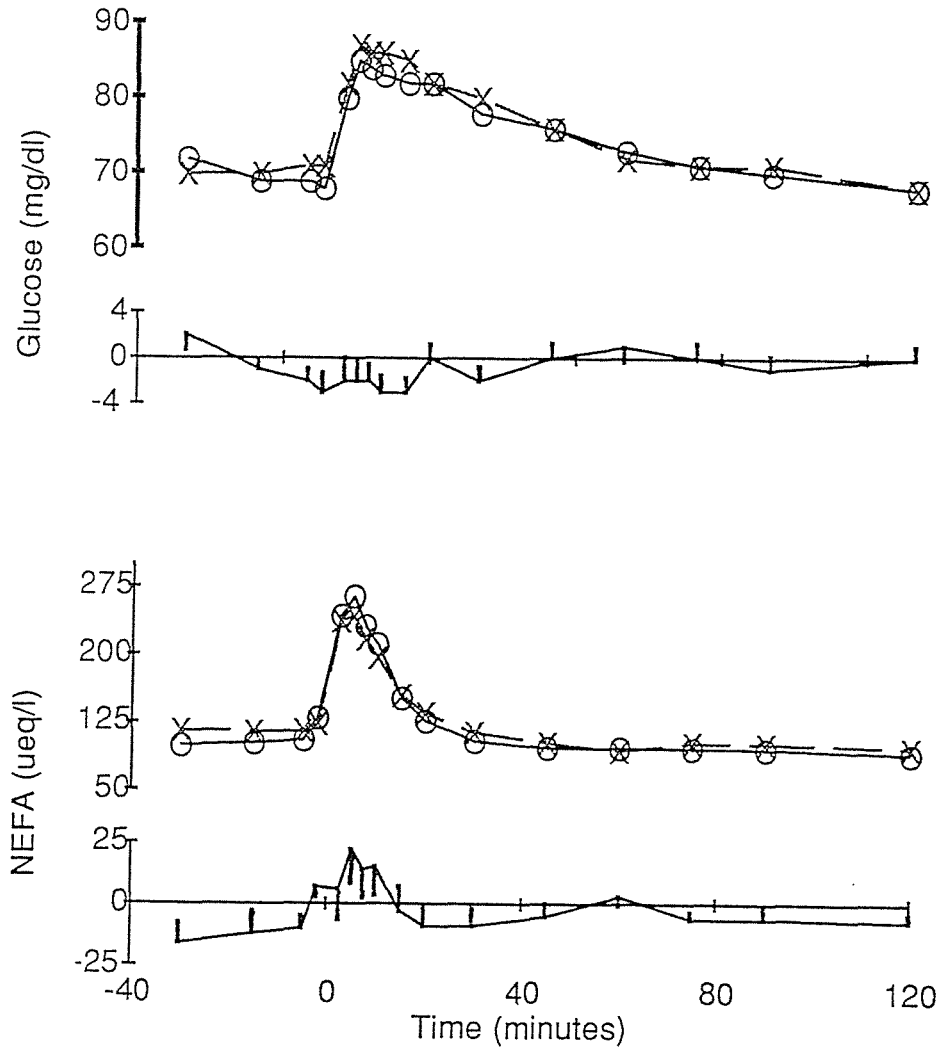

Figure 2.1 Plasma concentrations of glucose and non-esterified fatty acids during an intravenous adrenalin challenge (1.0μg/kg liveweight administered at time zero) in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

4. Insulin challenge

The 0.01 mg/kg dose of insulin caused a relatively constant rate of decline in plasma glucose concentration between 2.5 and 30 minutes following the injection. (Figure 2.2). There were no significant line or sire-within-line differences in either the rate of decline to 30 minutes or the rate at which glucose concentration rose after 30 minutes. NEFA concentrations rose slightly in the pre-challenge period, declined in response to the injection, and from 30 minutes after injection rose steadily to levels higher than baseline levels. (Figure 2.2). The rate of increase was higher in the meaty line, but not significantly so ($P>0.1$).

5. Glucagon Challenge

Plasma concentrations of glucose, NEFA and insulin in response to the 0.175 μg/kg glucagon challenge are presented in Figure 2.3. Glucose and insulin concentrations peaked at approximately 5 minutes after the injection. Between-line differences were not detected at any time in glucose or insulin concentrations following the injection. However the effect of sire-within-line on glucose concentration became significant in the interval 10 to 60 minutes after the glucagon injection. This effect was caused by one Meaty sire group whose glucose concentrations were significantly higher than those of the other sire groups. Plasma NEFA concentrations peaked at approximately 7.5 minutes following the injection. The response of meaty line above baseline levels (mean of the -30,-15 and -5 minute samples) diverged from that of the fat line from the -2 minute sample and was significantly higher during the interval 7.5 to 20 minutes ($P<0.01$).

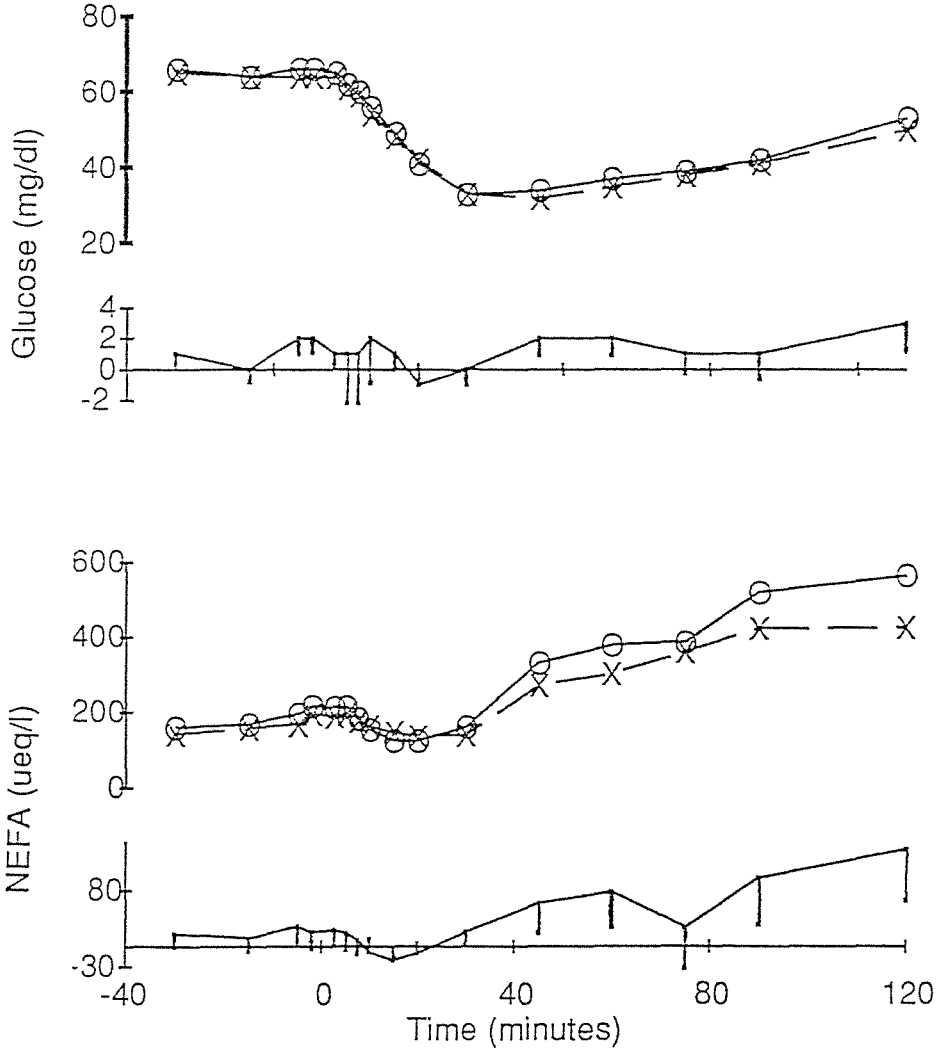

Figure 2.2 Plasma concentrations of glucose and non-esterified fatty acids during an intravenous insulin challenge (0.01mg/kg liveweight administered at time zero) in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

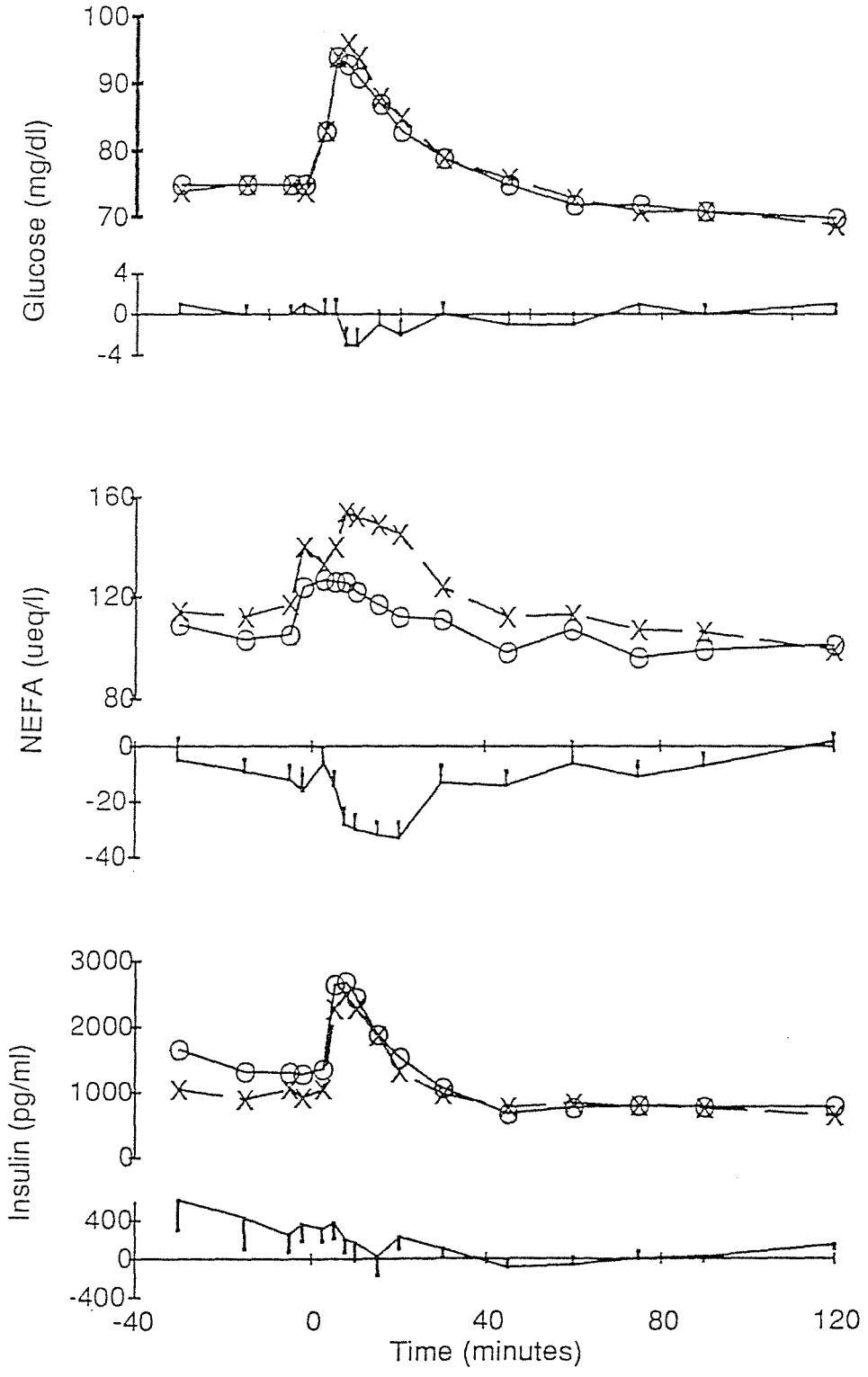

Figure 2.3 Plasma concentrations of glucose, non-esterified fatty acids and insulin during an intravenous glucagon challenge (0.175µg/kg liveweight administered at time zero) in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

6. Glucose Challenge

Plasma concentrations of glucose and insulin in response to the 0.17g/kg dose of glucose are shown in Figure 2.4. There were no significant selection line differences in the baseline concentrations of glucose but the fat line animals had significantly higher glucose concentrations in the interval 2.5 to 7.5 minutes post-injection ($p<0.05$). The mean glucose fractional decay constants (K_g), intercept values ($G_{(0)}$) and glucose distribution spaces as calculated over the period 5-30 minutes following the challenge are presented in Table 2.5. The fat line had a significantly higher mean $G_{(0)}$ and K_g values and a lower mean distribution space than the meaty line. The fat line tended to have higher baseline insulin concentrations ($p=0.06$) and insulin response above baseline levels in the period 5-15 minutes following the challenge ($P=0.08$).

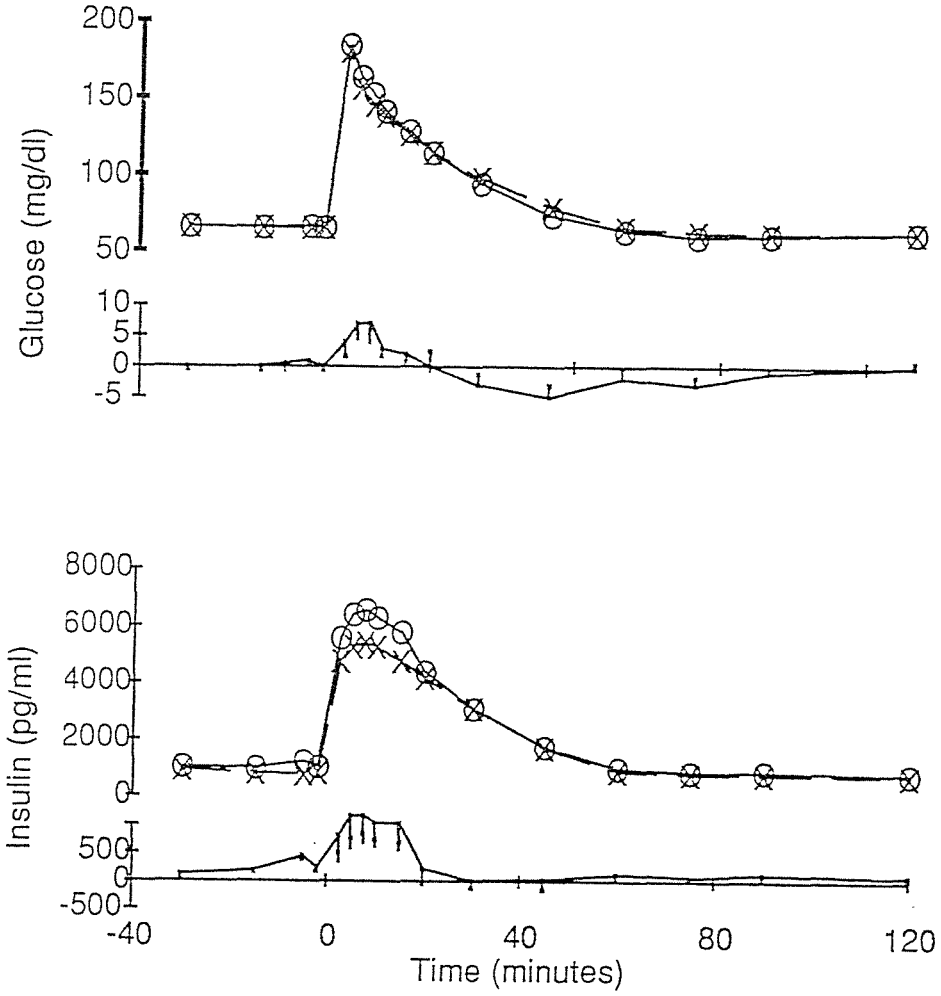

Figure 2.4 Plasma concentrations of glucose and insulin during an intravenous glucose challenge (1.7g/kg liveweight administered at time zero) in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

Table 2.4 Glucose tolerance test parameters calculated from plasma concentrations of glucose and insulin after an intravenous glucose challenge (1.7g/kg liveweight) in 10 Fat and 10 Meaty Southdown ram lambs

	Selection Line			
	Fat	Meaty	SED[a]	Significance
$G_{(0)}$ (mg%)	193	178	4	**
K_g (min^{-1})	-0.050	-0.042	0.003	*
Space (%)[a]	14.0	15.7	0.4	**
Insulin Res.[c] (min.pg/ml $X10_3$)	16.7	15.3	1.4	NS

[a] glucose distribution space as a proportion of fasted weight
[b] standard error of the difference
[c] insulin resistance calculated from glucose challenge data

Between-line variation in peak insulin concentrations was partly explained by between-line variation in glucose distribution space (r=-0.63). The glucose relative disappearance constant (K_g) was in turn significantly ($p<0.01$) related to peak insulin concentrations (r=-0.60). Between-line differences in K_g were non-significant when fitted after peak insulin or glucose distribution space. The between-line difference in the calculated insulin resistance values was not significant ($P>0.1$). The glucose distribution space was significantly ($P<0.05$) correlated with subcutaneous backfat measurement (r=0.47).

7. Fasting and Refeeding

The between-line difference in baseline growth hormone levels increased with fasting and was significant in the interval 24 to 51.5 h after the start of the fasting period ($P<0.05$). Growth hormone concentrations of both lines increased in response to refeeding. The magnitude of the response above fasting

concentrations in the Meaty line was approximately 3 times that of the Fat line. Differences were significant ($P<0.02$) at sample times 1.5 and 3.5 hours after refeeding (Figure 2.5). Urea concentrations of both selection lines rose by approximately 1.5 mM over the fasting-refeeding period. The significant between-line difference in baseline urea concentrations was maintained during both the fasting and refeeding periods (Figure 2.5).

Plasma glucose and insulin concentrations declined over the fasting period and both rose in response to feeding. NEFA and β-hydroxy butyrate concentrations rose over the fasting period, and dropped sharply in response to feeding (Figure 2.5). No significant between line differences were detected in glucose, insulin, NEFA or β-hydroxy butyrate concentrations at any sampling time ($P>0.10$). There was a significant effect of sire within selection line on glucose concentrations in the 39.5 to 71.5 h interval. This was largely a result of the progeny of one meaty sire maintaining significantly ($P<0.05$) lower glucose concentrations than progeny of the other three sire groups.

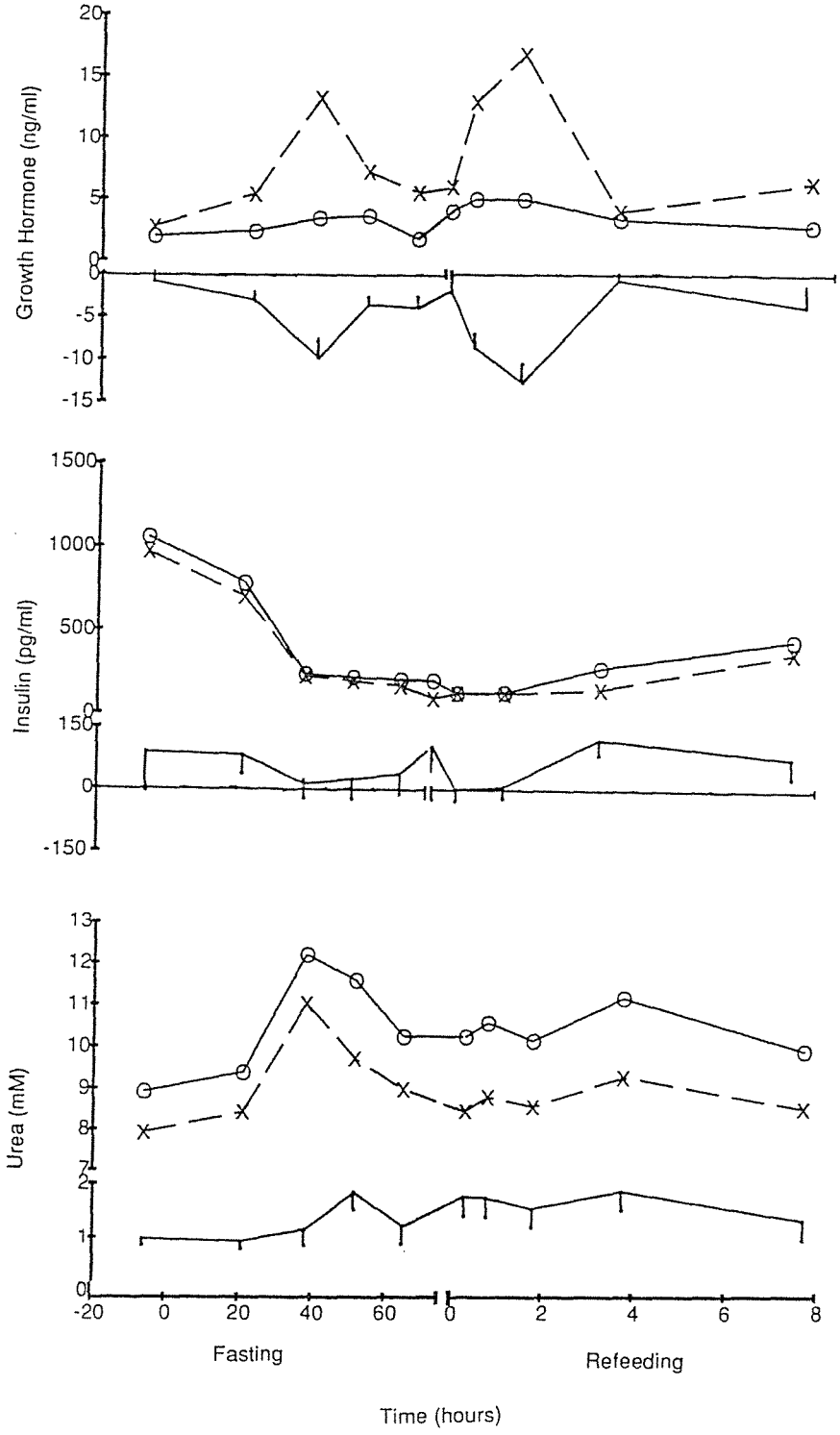

Figure 2.5 Plasma concentrations of growth hormone, insulin and urea during a 63.5 h fasting and 7.5 h refeeding period in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

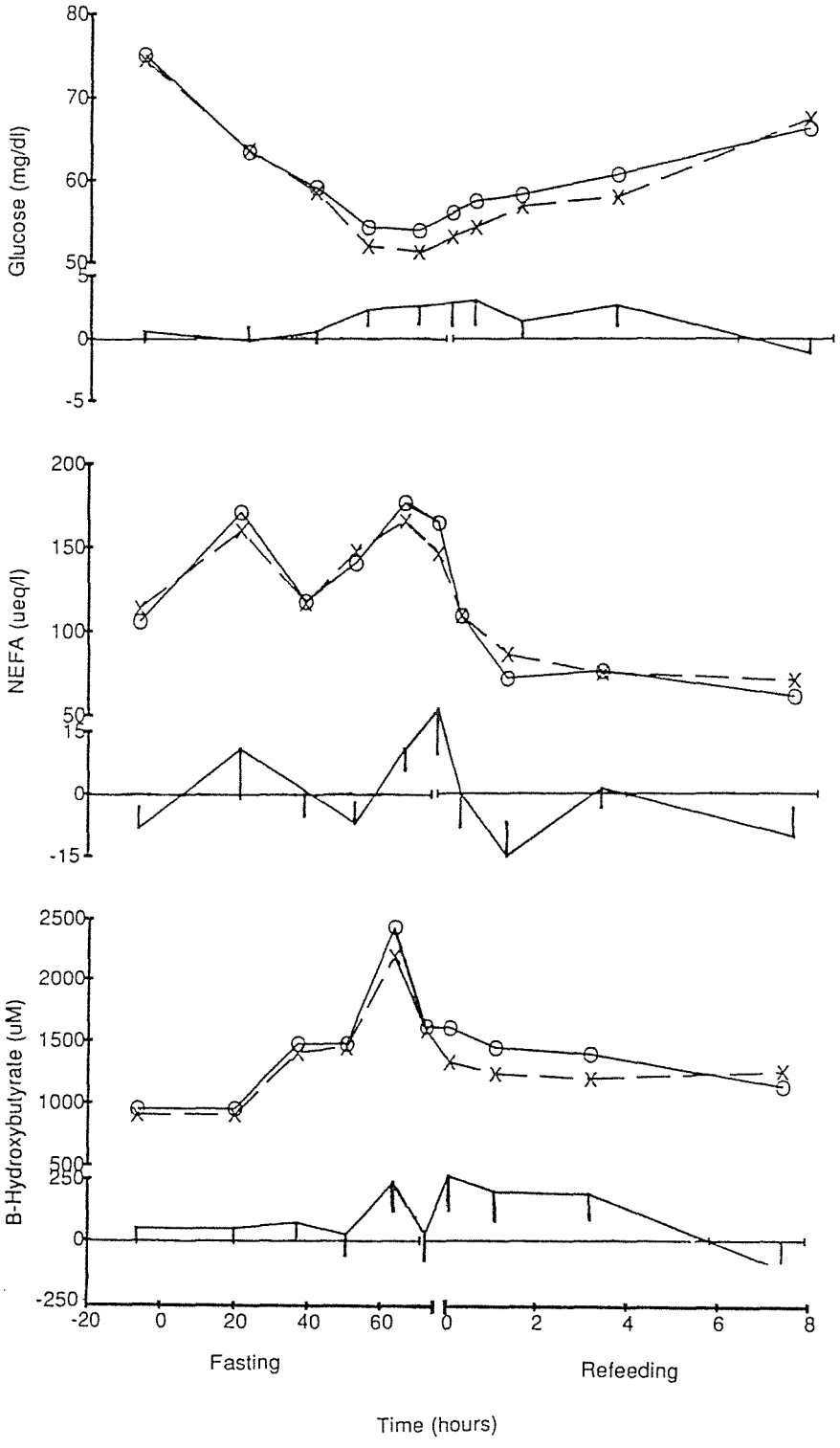

Figure 2.6 Plasma concentrations of glucose, NEFA and β-hydroxy butyrate during a 63.5 h fasting and 7.5 h refeeding period in 10 Fat (o) and 10 Meaty (x) Southdown ram lambs together with the difference (Fat - Meaty) and its SE (vertical bars)

Discussion

The results of this experiment suggest that baseline concentrations of some metabolites and hormones may be associated with genetic merit for lean meat growth and that homeorhetic control of metabolism may differ between the Fat and Meaty selection lines. Although there were no significant between-line differences in baseline concentrations of growth hormone, the difference in concentrations increased and became significant over the fasting and refeeding periods. A higher growth hormone concentration in the Meaty line is consistent with results showing higher growth hormone levels in lean, fast growing swine selection lines compared with contemporary fat, slow growing genotypes (Ringberg Lund-Larsen et al. 1975; Althen et al. 1976; Wangsness et al. 1977). Baseline growth hormone concentrations showed a low non-significant correlation with weight gain from birth to the time of the experiment. Previous work with growth hormone concentrations based on low numbers of samples taken with long intervening periods has also showed inconsistent correlations with growth rates (Trenkle et al. 1971; Hafs et al. 1971). It appears that correlations with growth rate are improved if sampling is carried out more frequently so that spike frequency and amplitude can also be estimated and used in a combined predictive model of growth rate (Klindt et al. 1985).

There was no between-line difference in IGF-1 concentrations shown in this experiment. This is in contrast with higher levels of IGF-1 concentrations shown in lean fast growing selection lines of pigs (Ringberg Lund-Larsen et al. 1975; Althen et al. 1976). The

difference in IGF-1 concentrations shown in those lines as compared to the present study may be related to the fact that the swine experiments also included growth rate as a selection criterion. Selection for low backfat, in the experiment from which the animals used in this study were drawn, has not resulted in correlated increases in growth rate (Kadim 1987). Baseline IGF-1 concentrations were significantly correlated with growth rate in this experiment. Similar observations have been made in sheep (Olsen et al. 1981) and in cattle (Ringberg Lund-Larsen et al. 1977). Although these studies do not necessarily indicate a genetic relationship between IGF-1 concentration and growth rate, such a relationship is supported by differences observed between swine selected for high vs low growth rates (Ringberg Lund-Larsen et al. 1975) and by correlated increases in growth rate shown in mice divergently selected on the basis of plasma IGF-1 concentrations (Blair et al. 1987). It therefore appears that IGF-1 may have potential as a marker of genotype for early growth rate.

The Fat line maintained plasma urea concentrations consistently higher than those of the Meaty line over the fasting and refeeding periods. Similar differences in blood urea concentrations between these lines at feeding levels above maintenance have subsequently been reported by Bremmers et al. (1987) and van Maanen et al. (1987). Furthermore, lower blood urea concentrations have been reported in genetically superior dairy cattle (Sejrsen et al. 1984; Sinnet-Smith et al. 1987), fleece weight-selected sheep (Clark et al. 1987; McCutcheon et al. 1987) and in pigs selected for lean growth (Mersmann et al. 1984). It

appears that plasma urea concentration may be correlated with genetic merit for a number of traits and therefore have potential as a genetic marker. However this relationship might only be evident under standardized conditions as it has been demonstrated that differences between the lines are diminished at low feeding levels (Bremmers et al. 1987) and under field grazing conditions (McCutcheon et al. 1987).

The principal source of urea is from liver conversion of ammonia produced by the deamination of surplus amino acids and rumen degradation of dietary protein. Mersmann et al. (1984) suggested that differences in plasma urea concentrations observed between lean and fat selection lines of pigs occurred as a result of more efficient use of amino acids for protein synthesis, and consequent reduced requirement to deaminate amino acids in the lean line. Alternative explanations for differences in plasma urea concentrations observed between selection lines are that urea excretion rate and/or the urea distribution space differ between the lines. Kidney excretion of urea is principally by passive diffusion and is therefore a function of glomerular filtration rate. Experiments carried out by Bremmers et al. (1987) and McCutcheon et al. (1987) with Meaty and Fat Southdown rams, and fleece weight-selected and control Romney rams, respectively, have failed to show between-line differences in urea excretion rate or creatinine clearance rate (indicative of glomerular filtration rate). However plasma urea differences between lines in those studies were non-significant when corrected to a common creatinine clearance rate and urea excretion rate.

Plasma concentrations of glucose and insulin decreased over the fasting period, and rose rapidly in response to refeeding. No significant between-line differences were detected although one meaty sire group maintained significantly lower glucose concentrations over the fasting period. This difference was not related to sire group differences in backfat depth, although this was the same group which had significantly higher glucose concentrations in response to the glucagon challenge. NEFA concentrations rose over the fasting period, and there was a corresponding increase in β-hydroxy butyrate concentrations. These responses are consistent with the animals conserving glucose, and meeting energy demands by the mobilization and catabolism of fatty acids and concomitant production of ketone bodies.

Rams of the Meaty line were more sensitive to lipolytic effects of glucagon, as indicated by their greater elevation of plasma NEFA concentrations in response to the intravenous injection of this hormone. A similar between-line difference was not evident in response to the adrenalin challenge. This is in contrast with work carried out with fat, slow growing selection lines of pigs which have been shown to be less sensitive to adrenalin than lean fast growing lines (Standal et al. 1973,1979; Mersmann 1985; Brocklen et al. 1986). Between-line differences in response to glucagon injections were not repeated in a subsequent study with rams also drawn from the Massey University Fat and Meaty selection lines (Morgan 1987). However in that case rams were subdivided into feeding levels above and below maintenance and a similar (but non-significant) difference existed only at the high feeding level.

Cellular response to both glucagon and adrenalin is mediated via the c-AMP cascade system (Hu et al. 1987). The fact that between-line differences were apparent in response to the glucagon challenge, but not to the adrenalin challenge, may imply that differences in sensitivity to glucagon occurred because of differences in glucagon receptor numbers and/or binding affinity.

Differences in pancreatic sensitivity to glucose or tissue sensitivity to insulin did not appear to exist between the Fat and Meaty lines. Lord (1986) was also unable to detect differences in glucose-insulin status of lean and fat Coopworth selection lines. Differences in the plasma levels of glucose and insulin in response to the glucose challenge appear to have been largely a result of between-line differences in glucose distribution space. The physiological basis for this difference in glucose distribution space is not clear. However one possibility is that this difference is related to between-line variation in total interstitial space, which may also explain between-line variation in plasma urea concentration.

In conclusion the significant between-line differences revealed in this study suggest that baseline concentrations of metabolites/hormones, and responsiveness to metabolic challenges, potentially offer a useful means of predicting genetic merit for lean meat production in young sheep. Further research is required to establish the genetic association between these parameters and merit for lean meat growth. There is also a need to determine the

conditions under which these parameters are best measured for predictive purposes. A greater understanding of the physiological basis of the differences observed in this study may also lead to development of more repeatable and less intensive sampling strategies for measuring these parameters.

CHAPTER 3

GENERAL DISCUSSION

Genetically divergent lines arising from selection experiments have been widely used to investigate the possibility that genetic variation in the traits of interest may be recognizable in terms of physiological characteristics. The basic assumption made is that although the genetic merit (in this case for lean meat production) of individual animals selected from the lines is not known, there is a substantial between-line difference in average genetic merit. Genetic relationships between the composition of growth and physiological characteristics are thus implied by the presence of between-line differences in the measured physiological parameters. This allows potential markers to be identified without the need, at least in the early stages of the research programme, to accurately estimate the genetic merit of individual animals. Identification of factors indicative of such physiological differences may provide better markers of genetic merit in that they may be less affected by environmental effects than the expression and/or measurement of the trait itself.

Although selection lines are useful in studies of this type, it is important to recognise their limitations. Such lines are generally established within a breed, starting with a limited initial base population. Therefore genetic variation exploited within lines in a particular experiment may not necessarily apply to other similar experiments, or to the dominant source of genetic

variation which exists in the population as a whole (either within a particular breed, or within the ovine species). Furthermore, because the costs of maintaining domestic animal selection lines are very high, numbers of animals per line and replication of lines are usually kept to a minimum. Selection lines are thus subject to effects of random genetic drift which cannot be accurately estimated. Genetic drift effects are compounded by the fact that the attainment of selection lines exhibiting marked phenotypic divergence requires many years of selection, response to selection in domestic animals being slow (usually less than 3% per year). The implication of this is that between-line differences may arise as a result of random genetic effects independent of the applied selection pressure. An example of this has been shown in an experiment where sheep selected for greasy fleece weight were found to also exhibit correlated changes in red blood cell haemoglobin type gene frequencies (Pijls et al. 1987). This effect was apparently independent of the fleece weight response to selection and could be accounted for by the effects of genetic drift. Another possibility is that the differences observed between selection lines are genetically linked but may not necessarily be involved with the control of metabolic processes which lead to variation in the trait of interest. In such a case selection on the basis of the linked factor would not necessarily lead to correlated changes in the composition of growth.

Also related to the availability of resources is the problem that physiological comparisons of selection lines are subject to random sampling errors as a result of testing only small numbers of

animals from each of the lines. This is a particular problem where the measurement of physiological parameters is time-consuming or expensive. Furthermore, animals studied within any one generation will commonly be the progeny of a very limited number of sires. Differences shown between lines may therefore result from chance sire effects. The most extreme example of this situation is where progeny of only one sire from each genetic group are used in a comparison. For example Sejrsen et al. (1984) examined metabolic characteristics of the progeny from each of one high and one low genetic merit dairy bull. Differences in plasma hormone concentrations were shown between the progeny groups but these might well have resulted from a peculiarity of the particular sires used, independent of their genetic merit for milk production. In such situations it is important that experiments be replicated in succeeding generations. Between-line differences in plasma urea concentration shown in the present study have since been supported by similar differences being observed in further exeriments involving two subsequent generations of rams (Bremmers et al. 1987; van Maanen et al. 1987). Under these cirumstances it seems unlikely that the between-line difference has occurred through chance sire effects.

Another potential difficulty in attempts to identify between-line differences in physiological parameters is that if an alteration in growth pattern is the product of small changes in many metabolic pathways, the ability to measure this variation in any one pathway may be beyond the accuracy of current assay techniques. In addition, even if differences are detected, they may only have low

genetic correlations with the composition of growth. Thus index approaches, involving the simultaneous measurement of many pathways, are likely to be required.

Despite these limitations there have been numerous attempts to identify physiological differences between lines of pigs, sheep and dairy cattle selected for body composition, wool production and yield of milk or milk fat. Physiological parameters examined can be broadly classified as 1) baseline concentrations of metabolites/hormones, 2) sensitivity to exogenous metabolites/hormones (challenges) and 3) enzyme activity levels in tissues.

Baseline Concentrations of Metabolites/Hormones

Measurement of baseline concentrations is a common first approach to studies into the basis of physiological differences which exist between selection lines. Measurement of baseline concentrations of metabolites or hormones has the advantage of relative simplicity, requiring only venous cannulation to obtain periodic blood samples. Cannulation has generally been adopted to avoid stress-related reactions which may occur with venipuncture blood sampling. However, it appears that with some metabolites/hormones (eg. IGF-1), cannulation is not required as concentrations are relatively insensitive to temporary environmental effects. The rationale behind the use of baseline concentrations of metabolites as predictors of genotype is that they may relate directly to the expression of the productive trait in that they are

intermediary metabolites in a pathway synthesising the productive tissue/metabolite of interest. Similarly, baseline hormone levels may reflect the level of endocrine stimulus provided to a pathway which affects the rate of growth of a particular tissue. The plasma concentration may be a function of the rate of turnover in the plasma pool, the size of the pool in which the metabolite/hormone is distributed, or the animal's set point at which modified levels of clearance and/or secretion are invoked.

Previous research has shown that non-genetic effects can interact with genetic effects on baseline concentrations of metabolites/hormones. These non-genetic effects include :

(a) Diurnal variation, which may be related to feeding patterns and/or photoperiod effects. For example a study carried out by Xing (1987) demonstrated a less marked diurnal pattern in plasma urea concentration in low genetic merit dairy bulls compared with bulls of high genetic merit. The magnitude of the between-group difference therefore varied with sampling time. To predict genetic merit on the basis of such a metabolite may thus require a standardised feeding and sampling programme to maximise accuracy. This would detract from the practicality of commercial application of such a marker.

(b) Energy balance has been shown to affect the magnitude of between-line differences in metabolite/hormone concentrations. Bremmers et al. (1987) have shown that the Fat and Meaty lines of interest in this study exhibited differences in baseline levels of urea only at a feeding level above maintenance.

(c) Genetically-based differences in concentrations of metabolites/hormones have been shown to change with age, and

possibly with degree of development relative to puberty. Van Maanen et al. (1987) demonstrated that plasma urea concentrations in the Massey Unversity Fat and Meaty lines of Southdown sheep converged as the lambs aged.

Standardisation of experimental conditions should be employed to minimize these effects but relies on certain assumptions which may not always hold. For example, attempts to minimize variation in energy balance commonly involve feeding animals to a fixed proportion of maintenance, with the added assumption that maintenance is proportional to metabolic weight. However, if maintenance requirement per unit metabolic weight varies between lines, such has been shown in fat and lean pig selection lines (Yen et al. 1983), actual energy balance may not be the same in the two groups. In this case physiological differences which appear between the lines may in fact be due to differences in energy balance. A more extreme example comes from the study of Hart et al. (1978) who compared basal hormone and metabolite concentrations in high and low producing dairy cows fed at similar energy intakes. Differences between the groups in hormone/metabolite concentrations were largely due to differences in energy balance (ie effects of energy balance and genetic merit were confounded) and disappeared when animals were fed to maintain equal energy status (Hart 1983).

Two further problems are associated with measurement and interpretation of baseline hormone concentrations. First, some hormones (for example growth hormone, Klindt et al. 1985) are released in pulsatile secretions, their effect on metabolism

apparently being a function of spike frequency, spike amplitude and average baseline levels. In this situation measurement of such hormones may require intense blood sampling over a long period of time if it is to have any predictive value. Second, the metabolic stimulus provided by any one hormone is markedly affected by the orchestrated interaction with many other hormones/metabolites. For example, the co-presence of insulin and thyroid hormones is required for *in vivo* growth hormone/IGF-1 stimulation of protein synthesis (Goldberg *et al.* 1980)). This suggests that ratios of relevant hormones may provide better indicators of endocrine status than does the measurement of any one hormone concentration.

Baseline concentrations of metabolites/hormones can provide useful information concerning the physiological basis of between-line differences. However, they yield no information as to the sensitivity of animals to particular metabolites or hormones. Alternative techniques, such as the use of acute intravenous challenges, are therefore being increasingly adopted.

Responses to Metabolic/Hormonal Challenges

In contrast to the measurement of baseline metabolite or hormone concentrations, intravenous challenges are used to detect differences between genetic groups in sensitivity to homeostatic signals. Interest in this approach has been generated by the proposal that animals achieve dynamic control of nutrient partitioning by regulating the sensitivity of specific target tissues to homeostatic regulators (Bauman *et al.* 1980). The previous

discussion has highlighted some of the evidence in support of this concept. Challenges of this type generally involve the measurement of baseline metabolites/hormones prior to administration of the challenge, and of changes in concentrations following the challenge until they return to or approach baseline levels. The difficulty with this approach is that there will generally be multiple mechanisms operating to counteract the effects of an administered challenge (ie to maitain the homeostatic balance). Related to this is the fact that if a challenge produces a larger response in one particular group, these animals may produce a larger counteracting response. The implication is that response curves will tend to converge as the challenge proceeds and, if differences exist, they will tend to be most evident early in the response period.

Response to challenges may be evaluated in a number of ways. Where challenges alter plasma concentrations of metabolites/hormones, peak concentrations or integrated responses (area under the response curve) are typically used. The magnitude of these may be a function of altered secretion rate, clearance rate and/or differences in pool size. An example of this can be seen in response to the glucose challenge in the present study. Animals of the Fat line showed higher initial glucose concentrations which were probably related to differences in glucose distribution space. Over the same period the Fat line also exhibited higher insulin concentrations which may have invoked a higher level of glucose clearance. Higher insulin concentrations could also have resulted from differences either in distribution space or in the rate of secretion/clearance.

Evaluation for the purpose of identifying possible markers of genetic merit, while not attempting to make inference about the physiological basis of the response, may simply require mean plasma concentrations of metabolites/hormones to be taken over selected intervals following the challenge. Enhancements to this approach include response correction for different base-line (pre-challenge) concentrations and selection of the intervals over which the between-line variation is maximized and/or within line variation minimized. From a physiological point of view it is desirable to relate response to rates of production vs clearance such that quantitive estimates of sensitivity of the various control mechanisms can be estimated. For example, to evaluate the response to the glucose challenge, it would be desirable to estimate pancreatic sensitivity to the glucose load and peripheral tissue and hepatic sensitivity to the induced insulin secretion. Bergman et al. (1981) has modelled the response to a glucose tolerance test in dogs in terms of the simultaneous effects of glucose on insulin secretion, and insulin on hepatic glucose synthesis and glucose dissappearence into peripheral cells. The problem with this approach is that the effects of individual response mechanisms on plasma concentrations are highly correlated. Therefore with low numbers of data points it becomes statistically impossible to estimate these parameters.

Responses to challenges, like baseline concentrations, are subject to non-genetic effects and interactions of these with genetically correlated traits. Morgan (1987) has shown that

differences between genotypes in response to challenges are subject to effects of energy balance. For example, a significant interaction was shown between selection line (Fat vs Meaty Southdowns) and feeding level in the response of plasma NEFA concentration to a glucagon challenge. In a study of the sensitivity of heifers to the glucoregulatory effects of insulin, McCann et al. (1985b) demonstrated that the difference between lean and obese heifers in response to insulin varied according to the stage of the oestrus cycle. Thus the magnitude of differences between groups in response to challenges may be influenced by a variety of non-genetic factors. It therefore becomes important to establish the optimum conditions under which challenge studies should be conducted.

Summary

Despite the problems associated with measurement of metabolic and hormonal factors, this study indicates that baseline concentrations, and response to some metabolic challenges, have potential to provide useful predictions of genetic merit for lean meat production. This is further supported by similar differences having been shown between a variety of other selection lines. Future research should now address the extent to which environmental effects bias the measurement of these factors and the conditions under which these factors are best measured. Research in this field may not only improve the genetic relationship of the measured parameter/s with genetic merit, but also indicate the basis of the mechanism by which genetic variation arises. True genetic relationships will also have to be determined between the potential

markers and actual genetic merit. This will require that genetic merit of individuals be determined (probably by progeny tests) and related to their ranking on the basis of physiological markers, or that realized genetic parameters be determined from selection experiments on the basis of the new marker. Only then will it be possible to make an economic appraisal of the potential gains which could result from the use of physiological markers in the commercial situation.

APPENDIX

Assay Methods for Hormones and Metabolites

1. Glucose

Glucose concentrations were determined using a YSI Model 27 industrial analyzer (Yellow Springs Instrument Co., Colorado). The method is based an electrode sensitive to hydrogen peroxide which is produced in an enzymatically catayzed oxidation of glucose. Samples are injected into a chamber where they are diluted in buffer and allowed to diffuse through a polycarbonate membrane in which the bound glucose oxidase enzyme resides and the reaction takes place.

The instrument was calibrated using six standards at concentrations of 0,25,50,75,100 and 150 mg/dl. Repeatibility of readings was checked by duplicate analysis of one of the standards after every five samples. The coefficient of variation between duplicate readings of the standard analysis was less than 1%.

2. Non-Esterified Fatty Acids

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 NEFA 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-β-hydroxyethyl-aniline

with 4-amino-antipyrine to form a purple complex, the optical density of which is measured at 550 nm. Standards are provided as oleic acid. Samples were assayed adopting the modified method of McCutcheon et al. (1986) which allowed a reduction of sample and reagent volume to reduce the assay cost. This modification was shown not to affect the accuracy. Intra- and inter-assay coefficients of variation were 3% and 10% respectively.

3. Urea

Plasma urea concentration was determined by the method described by Marsh et al. (1965). This is an automated colourimetric method where the combined use of thiosemicarbazide and ferric ions alters and intensifies the colour of a complex produced in a direct reaction between diacetyl monoxine and urea. Intra- and inter-assay coefficients of variation were 3% and 6% respectively.

4. Radioimmunoassays

Insulin and growth hormone were assayed using the double antibody radioimmunoassay method described by Flux et al. (1984). The source of the hormone used for standards and trace, linear range of the assay (log-logit transformed data) and intra- and inter-assay coefficients of variation are presented in table A.1. Iodination of the trace hormone was carried out by the chloramine-T method (Greenwood et al. 1963). The first antibodies, for both assays, were raised in guinea-pigs, and the antiserum to guinea-pig gamma globulin in a Romney sheep.

Table A.1 Source of hormone and assay parameters of the assays for the measurement of insulin and growth hormone concentrations

	Hormone Source		Assay Parameters		
Assay	Standards	Trace	Linear Range	Intra-Assay CV	Inter-Assay CV
Insulin	Bovine I-5500 Lot 121c-1350[a]	Bovine I-5500 Lot 121c-1350[a]	100-12800 pg/ml	8.2	12.4
Growth hormone	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.

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