Acetyl CoA Carboxylase, Adipocyte P2, Lipoprotein Lipase, and Hormone Sensitive Lipase mRNA levels in Ovine Adipose Tissues and Their Relationship with Carcass Fat

by

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DEDICATION

This thesis is dedicated to my wife, Doris. Her determined spirit and unwavering support through these long years gave me the strength to go on, even when I thought I'd never finish. It is also dedicated to my parents, Michael and Delia, who have taught me that success is not about what you have, but how you feel inside.

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ABSTRACT

The lack of an accurate predictor of carcass composition on live animals is a major limitation facing effective selection programs aimed at reducing fat content in sheep. Recent advancements in molecular biology have provided an opportunity to identify genetic markers that could facilitate identification of lean animals at an early age. The level of mRNA of genes controlling fat metabolism could be a useful source of information in choosing potential candidate genes. The objective of this study was to obtain preliminary data on acetyl co-A carboxylase (ACC), adipocyte P2 (aP2), hormone sensitive lipase (HSL), and lipoprotein lipase (LPL) as potential markers for carcass composition. Two experiments were performed. Karakul (n=5), Rideau Arcott (n=5), Dorset (n=5), Texel (n=3) and Texel-Dorset cross (n=5) animals were used in the first experiment, and represented substantial differences in carcass composition. The mRNA levels of the four genes were measured in the subcutaneous fat samples collected at slaughter. Texel (the leanest breed studied) had a lower unadjusted LPL mRNA value ($P<0.05$) than Dorset and Texel-Dorset cross lambs. LPL mRNA also showed positive correlations with percentages of subcutaneous fat ($r=0.39$, $P=0.06$) and total carcass fat ($r=0.42$, $P<0.05$), and negative correlations ($P<0.05$) with percentage of muscle ($r=-.49$), and ratios of muscle to total carcass fat ($r=-0.51$), subcutaneous fat ($r=-0.52$) and intermuscular fat ($r=-0.42$). ACC, aP2 and HSL mRNA levels did not show breed differences, nor did they show any significant correlation with carcass composition. In the second experiment, fat biopsies were taken from the tail of five Karakul lambs at monthly intervals from one to seven months of age to evaluate gene expression during growth. Growth of the fat depot was estimated by measuring length, thickness and circumference of the tail at the time of taking fat biopsies. In trial II, ACC and LPL mRNA exhibited significant changes over the monthly periods during growth and mirrored the nutritional status of the animal. Strong positive relationships ($P<0.05$) were detected when daily gains in tail thickness and circumference were regressed on LPL mRNA levels ($b=0.419$ and 0.366, respectively), explaining 7.7% of variations in each trait. Similar relationships were found between ACC mRNA and daily gains in tail thickness ($b=0.467$, $R^2=5.8\%$) and tail circumference ($b=0.482$, $R^2=8.1\%$). ACC and LPL mRNA levels were not related to body weight gain, actual measures of tail dimensions, or daily gain in tail length, where changes in the latter were more closely related to chronological age and skeletal growth than to the variation in the rate of fat accumulation in the tail. Adjusting mRNA of the genes for β-actin tended to increase the variability of the data, and reduced the number of significant differences in both trials. The results were, however, consistent with unadjusted mRNA values. The results of these two trials showed that LPL holds the most promise in the search for markers for carcass fat content. Although ACC was shown to play a pivotal role in fat metabolism in sheep, it may not be a proper candidate gene because of the lack of an association with carcass composition.

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1. INTRODUCTION

The efficient production of a lean carcass is an essential component for any meat animal industry (Berg and Walters 1983). Although carcass traits are highly heritable (Simm 1992) and thus would respond quickly to selection programs, there are no accurate estimators of carcass composition in the live lamb. The identification of DNA based markers for the estimation of carcass composition could provide the necessary information for the selection of lean, efficient animals at an early age. Identifying differences in the activity of candidate genes would provide information regarding a gene's potential use as a marker for selection.

Ruminant fat metabolism has been studied extensively, and key enzymes regulating the various pathways involved have been identified. Acetyl CoA carboxylase (ACC) is a key enzyme in the biosynthesis of fatty acids in the ruminant, and is an important determinant of a cell's potential to synthesize fatty acids (Vernon 1992). Lipoprotein lipase (LPL) cleaves triglycerides (TGs) from circulating lipoproteins in the blood, thus regulating the adipocyte's uptake of circulating TGs (Eckel 1989). The hormone sensitive lipase (HSL) is the rate-controlling enzyme in the mobilization of stored fatty acids within the adipocyte (Yeaman 1990). Together, these three enzymes control the rate of fat accreditation. The adipocyte P2 (aP2) gene codes for an adipocyte-specific, fatty acid binding protein involved in fatty acid trafficking within the cell (Matarese *et al.* 1989).

Limited information is available on the relationships between carcass composition and activities of these enzymes or the messenger RNA (mRNA) levels of the genes in

ruminant animals. Muggli-Cockett *et al.* (1992) has found strong correlations between aP2 and LPL mRNA levels and carcass characteristics in lambs of mixed-breeds, showing that mRNA levels of genes controlling fat metabolism may be indicators of carcass fatness in sheep.

The objectives of this research were (1) to estimate the relationships between mRNA levels of ACC, LPL, HSL and aP2 genes and carcass fat content, and (2) to study the relationships between mRNA levels of these genes and the rate of fat accumulation during the growth phase. This information will aid in the selection of candidate genes for the development of molecular markers. By identifying mRNA levels that parallel the development of adipose tissues, it would be possible to identify genes that play an important role in the regulation of fat content in the sheep. Genes showing the most promise can be studied in greater detail, identifying alleles that influence carcass composition. Presence of a significant relationship between mRNA of a gene and carcass composition, however, may not be due a mutation in the gene itself, but could be caused by a mutation in another gene (e.g. a transacting regulatory gene). Such differences could be the results of mutations or environmental factors, such as energy intake. Nonetheless, information on the associations between carcass fatness and mRNA of a gene is a logical starting point in searching for DNA markers for carcass fatness, which is controlled by a very large number of genes, as well as environmental factors. Two separate experiments were performed to meet these objectives. The first experiment investigated mRNA levels of ACC, aP2, HSL and LPL genes in five breeds that varied significantly in their carcass fat content. Differences between lean and fat breeds could indicate the importance of a gene. The second experiment involved the

investigation of the association between mRNA levels and the rate of fat accumulation during the growth phase. The fat tail Karakul sheep provides an excellent model to monitor the rate of fat accumulation and mRNA levels simultaneously, as the activity of a gene can be easily related to the size of the tail fat depot.

While monogastric *de novo* fatty acid synthesis occurs primarily in the liver, lipogenesis in the ruminant animal occurs within the adipocyte (Vernon 1992). This makes the ruminant animal a convenient model to study fat metabolism as lipogenesis and lipolysis occur simultaneously in the same tissue.

2. LITERATURE REVIEW

A. Lean Carcass Production

i. Reducing Fat Content

The consumption of red meat has steadily declined, mainly due to perceived health risks associated with the consumption of excess fat (Kempster *et al.* 1987). Nowhere is this more apparent than in the sheep industry, as Canadian consumers have identified fat content as the primary reason to avoid lamb products (Jeremiah *et al.* 1993). The production of lean meat must be emphasized, as excessive fat will severely hamper any effort to increase lamb consumption in Canada.

Excessive fat can also represent inefficiency in the animal production system. The production of fat tissue represents a considerable energy cost (Trenkle and Marple 1983) provided in the form of feed, and thus any fat in excess of consumer demand represents a considerable decrease in the production efficiency of the growing animal. The efficient

production of a carcass that satisfies consumer demands has been recognized, both internationally and locally, as an essential component for the survivability of the sheep industry (Berg and Walters 1983).

ii. Selection for Lean Meat Production

Carcass fat can be manipulated by several genetic and management methods. Slaughtering animals at an early age will reduce fat content (Kempster *et al.* 1987), but can also increase the cost of production per kilogram of saleable meat. Dietary manipulation has produced conflicting results, and its usefulness would be limited as the nutritional management of the sheep is largely determined by the production system (Butler-Hogg and Johnsson 1986, Beauchemin *et al.* 1995). Pharmacological additives (McLaughlin *et al.* 1993, Fennessy *et al.* 1990) are available, but negative conceptions associated with their use may further reduce consumer demand.

Crossbreeding between maternal- and sire-breeds has been widely utilized by commercial sheep producers as a method to produce lambs with desirable carcasses (Leymaster and Jenkins 1993). However, carcass traits do not show much heterosis (Simm 1992), so carcasses of crossbred lambs are only expected to be midway between those of the sire and dam breeds making up the cross. Therefore, the selection of lean animals within breeds remains an important issue. In general, carcass traits are moderately to highly heritable (Simm 1992). In recent review, Simm (1992) reported the heritabilities for carcass fat and backfat depth at a constant weight to be between 0.4-0.5 and 0.25-0.37 respectively, indicating that a rather high rate of genetic progress could be achieved by selection. The usefulness of genetic selection has been impeded by the development of an

accurate and economical measure of fat content in the lamb.

iii. Estimation of Carcass Composition

An accurate assessment of the carcass composition of live animals is essential for selection of lean, efficient animals. Present selection methods include visual appraisal, live weight at a constant age, and ultrasound scanning. Visual appraisal can be quite subjective, and its usefulness is reduced as selection progressively reduces variation between animals. While some suggest live weight, when adjusted for breed and sex, is a valuable measure of carcass composition (Simm 1992), it was found that selected lean and fat lines within a breed had a difference of less than 2% in carcass weight (Cameron 1992), suggesting lean deposition can be independent of growth rate or live weight.

Ultrasound has been used to estimate fat content in swine, but limited success has been realized in beef cattle (Houghton and Turlington 1992). A recent review of the application of this technique to sheep found considerable variation in the accuracy of ultrasound, and concluded future application of this technique will depend on the development of an easy, rapid measurement that requires little interpretation by the operator (Houghton and Turlington 1992). Ultrasound techniques can not discriminate between small differences in tissue depths (Houghton and Turlington 1992). This reduces the effectiveness of the ultrasound, as most farmers use visual appraisal and body weight to preselect animals, effectively reducing the range of backfat thickness seen between animals. Furthermore, the usefulness of this measurement would be reduced as progressive selection would reduce the natural variation in backfat tissue depth and loin eye muscle dimensions.

The direct measurement of carcass composition cannot be performed on live animals. Simm (1992) suggests that semen and embryos collected before slaughter could act as a genetic reserve, for use after a carcass dissection was performed. This seems impractical and expensive under present conditions, although it may become feasible in the future. Progeny testing, used in other farm animals, would also seem impractical given the present level of reproductive technology in the sheep industry and the costs associated with carcass dissection (Simm 1992).

B. Application of Molecular Genetics

i. Introduction and Background

Tissue development and growth are controlled by processes that occur at the cellular level (Trenkle and Marple 1983), and it is these cellular processes, dependent on both environmental and genetic signals, that control the overall fat content of the animal. The goal of the animal breeder is to separate the genetic component from the environment, thus providing an accurate assessment of an individual's breeding value.

Historically, the animal breeder has had only phenotype to assess breeding potential. However, recent advancements in molecular genetics have provided animal breeders with a new set of tools, allowing access to the extensive genetic polymorphisms at the DNA level. Some of these polymorphisms control the genetic component of phenotypic variation, and the ability to dissect and uncode animal genomes will allow us to produce a direct measure of an animal's breeding potential. This information would be available at birth, providing a faster, more accurate assessment of traits that are sex limited, expressed later in life, and those that are difficult to measure, such as carcass composition.

Currently, the challenge is to identify these key sequences of DNA controlling economically important traits. Two main approaches in the search for DNA markers for genetic improvement of economically important traits include the use of microsatellite markers in combination with species linkage maps and the investigation of candidate genes.

ii. Evenly Distributed Anonymous Microsatellite Markers

This approach requires a basic framework of information before it can be used effectively. First, a method to effectively "tag" or "mark" the economically important sequence is needed. Microsatellites, small segments of di-, tri- or, tetranucleotide repeats that can be repeated up to one hundred times, are the markers of choice. They have been chosen as markers because they are highly polymorphic, and therefore show high levels of heterozygosity. These genes are numerous, distributed relatively evenly throughout the genome, are inherited in a Mendelian fashion showing codominance, and are easily identified by the polymerase chain reaction (PCR) using a small amount of DNA (Tautz 1989).

Secondly, microsatellites must be mapped to a specific location on a chromosome using a variety of statistical and molecular techniques. The current genome map for sheep contains 500 microsatellite markers, with between 8-48 markers available per chromosome (Freking 1996). This comprehensive linkage map allows researchers to scan the genome with these evenly spaced markers to identify areas of the genome linked to economically important traits. Once an area of interest has been established, a more comprehensive scan of the target area is also possible, thus narrowing the distance on the chromosome between

the marker and the actual gene of interest. This method has been successfully used to identify areas in the porcine genome which appear to be associated with fatness (Anderson *et al.* 1994), as well as the localization of the callipyge gene to chromosome 18 in the ovine genome (Cockett *et al.* 1994). Physical limitations of the system include the need for reference families (and the costs involved in establishing them), as well as the need for a large number of animals for the marker identification process. Other problems arise due to the distance between the marker and the causative polymorphism. If this distance is great, the association between marker and trait may erode over time. Therefore, there is a chance that the allele of interest will not be present if selection is based on the marker, leading to a reduced rate of genetic response due to inaccurate information (Smith and Smith 1993). Specific markers may only work within reference families or breeds, further complicating selection using microsatellite markers (Smith and Smith 1993, van Arendonk *et al.* 1994).

iii. Candidate Genes

Certain alleles of the key enzymes controlling metabolic pathways may have a pronounced effect on certain traits. Each allele may differ slightly in their expression; thus certain alleles may be more favorable for a specific production trait. The candidate gene approach involves the identification of such alleles with a major influence on traits of interest. The obvious disadvantage to the system is our limited knowledge of both the identity and the location of all genes controlling economically important traits. However, the accelerated speed by which new genes are identified and sequenced suggests this approach will become increasingly useful in the future. Several examples of gene markers

exist, including the estrogen gene marker affecting litter size, Major Histocompatability Complex (MHC) gene markers affecting health and reproduction, and an Insulin-like Growth Factor-1 (IGF-1) marker affecting growth rate (Rothchild 1995). A major advantage of the candidate gene approach is an increase in linkage disequilibrium between the marker and the trait of interest when compared to anonymous markers, providing a marker that should be applicable across families and should also resist erosion over generations (Smith and Smith 1993, van Arendonk *et al.* 1994). Searching for useful DNA markers is both expensive and time consuming, as a large number of genes must be screened, mutations must be identified, and the effect of these mutations established. Therefore, there is a need for information that will guide researchers, helping them to select a smaller sub-set of genes to perform detailed DNA analysis.

a. Identification of Markers Using mRNA Levels

Several researchers have used mRNA levels as the first in a series of steps to identify potential candidate genes (Muggli-Cockett *et al.* 1992, Brockmann *et al.* 1996). Messenger RNA forms a crucial link in the production of a functional protein, acting as an intermediate to transfer the genetic code held within the nuclear DNA to the cellular machinery within the cytoplasm for translation into protein. There are a series of events that occur between the transcription of a gene and the development of the functional protein (Figure 1). Control can be exerted at any step in the series: transcription, processing, transport into the cytoplasm, translation, degradation, or post-translational modification of the enzyme or protein (Alberts *et al.* 1994). Monitoring mRNA levels will allow us to identify a few candidate genes for detailed DNA analysis. A crucial assumption

in this approach is that any change in DNA that would elicit a change in phenotype should also change mRNA levels for this gene. In most cases transcriptional control has been shown to be paramount, most likely due to the biological inefficiencies of producing intermediates that would not contribute to the development of a functional protein (Alberts *et al.* 1994).

Assessing mRNA levels is a logical method of gathering information that could provide the preliminary evidence necessary to identify candidate genes that appear to play a regulatory role in carcass fat content.

C. Factors Affecting Carcass Fat Content

i. Adipose Tissue Structure and Function

A complex array of tissues comprises what is commonly known as adipose tissue. Slavin (1985) defines adipose tissue as a special type of connective tissue consisting mainly of specific lipid-laden cells (adipocytes) surrounded by a collagenous matrix containing nerves and blood vessels. The adipocyte itself is characterized by a spherical to oval shape, with a large, centrally located lipid droplet within the cell, and the nucleus is located peripherally as a flattened or pressed structure (Slavin 1985). While it was once thought that adipose tissue acted only as a storage for excess energy, it is now accepted that adipocytes are dynamic cells, with release and storage of fatty acids occurring continuously (Vernon 1980).

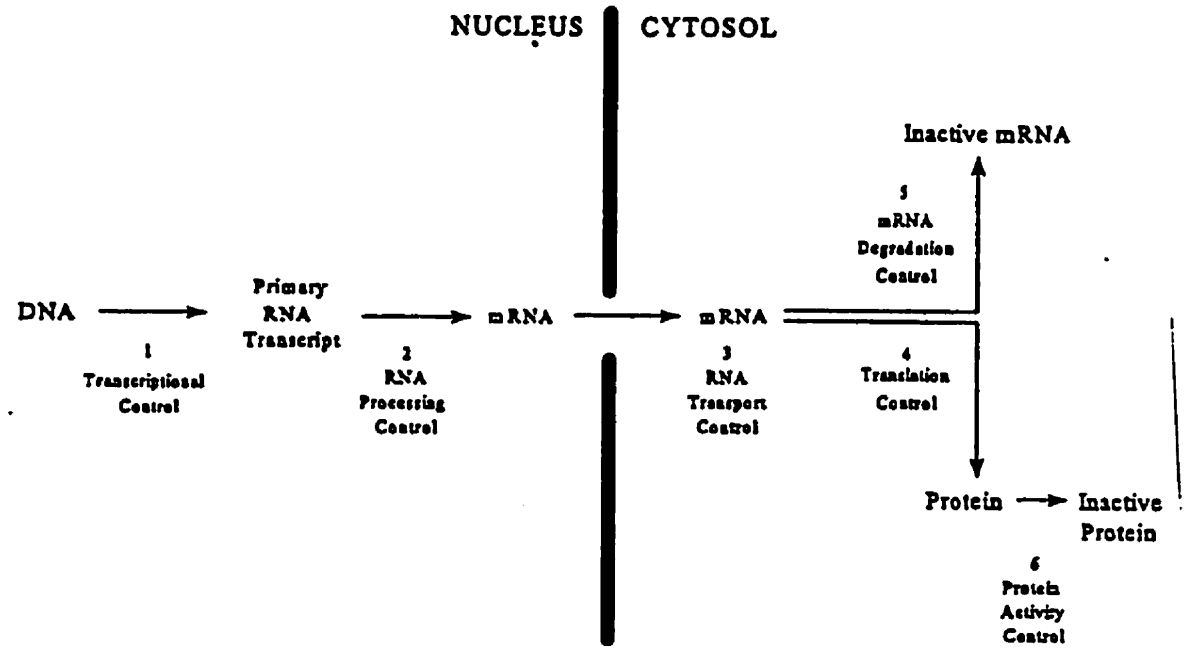

Figure 1. The control of protein production and activity

(Adapted from Alberts *et al.* 1994).

Adipocyte function (Figure 2) includes all aspects relating to the storage of lipid energy: the uptake of fatty acids, the synthesis and storage of lipid in the form of TG droplets, and the hydrolysis and release of free fatty acids stored in the form of triglycerol (Slavin 1985). On a cellular level, increases in adipose tissue mass are accomplished through two avenues, hyperplasia and hypertrophy (Van 1985). Hypertrophy within the fat cell is mainly due to the increase in stored lipid. Fat deposition occurs when the rate of

fatty acid esterification (storage) exceeds the rate of hydrolysis (mobilization) (Vernon 1980). Much information is available on hypertrophy and several key enzymes in this process have been identified.

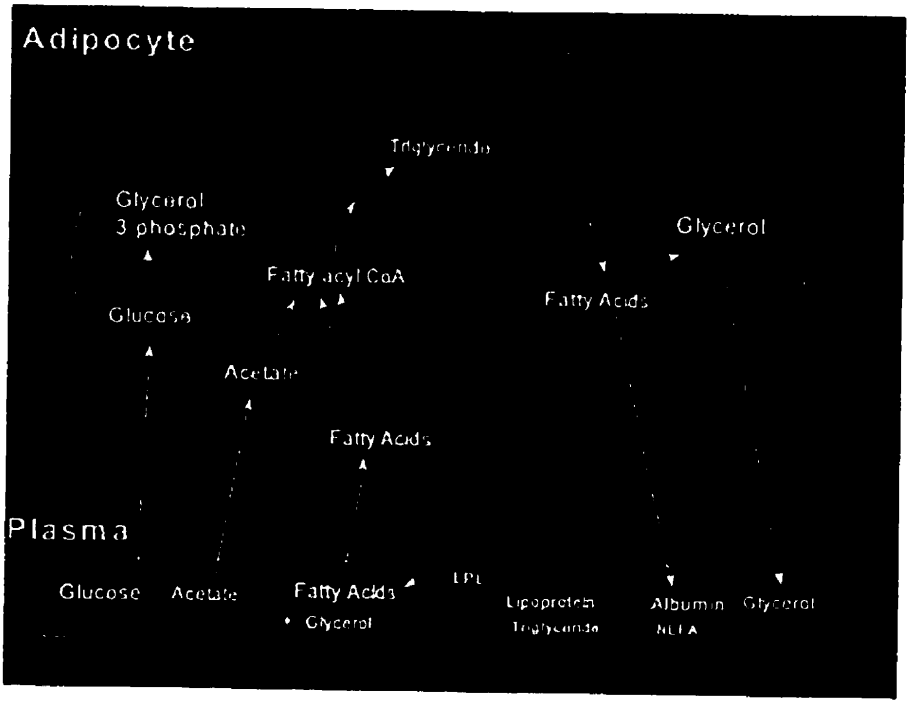

Figure 2. Activities within the Adipocyte (adapted from Vernon *et al.* 1980).

Hyperplasia, or an increase in cell numbers, also influences body fat content. While early beliefs were that cell numbers did not change after puberty, fibroblastic cells obtained from the stromal vascular fraction of adult human and rat adipose tissue were found to possess a preadipose nature, and could accumulate a large amount of lipid upon growth

arrest at confluence (Hiragun 1985). Hiragun (1985) cites this information as proof that "pre-adipocyte" obtained from adults still possessed the ability to proliferate. The proliferation and differentiation of pre-adipocyte is complex, and with only a few genes implicated in the process, much research is still required. Therefore, the current research has been limited to hypertrophy.

ii. Cell Hypertrophy

Before any attempt at locating potential genes can be made, a basic understanding of the processes involved is needed. Researchers have studied adipocyte fat metabolism extensively, identifying key areas in the regulation of both deposition and mobilization, and the following paragraphs will provide a brief summary of this research.

Lipid deposition is achieved by the storage of TG obtained from both circulating fatty acids and *de novo* fatty acid synthesis (Slavin 1985). Both processes require a source of glycerol-3-phosphate, as fatty acids must be esterified before being permanently stored in the fat droplet. Likewise, lipid mobilization is dependent on a series of signals that activate the enzyme pathway responsible for lipolysis (Vernon 1992). Esterification and lipolysis occur simultaneously and continuously within the cell, so there is a constant turnover of triglycerol, but the relative rate of these two processes determines if there is net loss or accumulation of stored lipid (Vernon 1992). Therefore the process of fat accreditation is regulated both by the activity of lipogenic and lipolytic enzymes within the system, and the concentrations of substrates and products available to the system (Vernon 1980).

a. Uptake of Circulating Triglycerides

Circulating TG uptake into the cell is regulated by LPL (Figure 2), which is produced within the adipocyte (Vernon 1992, Raynolds *et al.* 1990, Eckel 1989). Apolipoprotein-CII, a protein found on the surface of circulating high-density lipoproteins (HDLs), chylomicrons, and very low density lipoproteins (VLDLs), has been shown to activate LPL (Raynolds *et al.* 1990, Eckel 1989). The activated LPL then catalyzes the hydrolysis of circulating lipoprotein TGs (Vernon 1992), releasing free fatty acids (FFA) and monoglycerides (MG) from the lipoprotein. It has been suggested that the FFA and MG are transferred to the adipocyte by a continuous membrane leaflet extending from the surface film of the lipoprotein, through the endothelium and extracellular space, into the endoplasmic reticulum of the cell (Eckel 1989, Scow and Blachette-Mackie 1985). The monoglycerols are further reduced to FFA and glycerol within the adipose cell (Eckel 1989).

Since plasma TGs must be hydrolyzed prior to uptake into the cell, LPL effectively regulates the uptake of circulating fatty acids (Davey 1986, Eckel 1989, Vernon 1980). LPL plays a pivotal role in monogastric species, as it is the only pathway through which FAs can be transferred to the adipocyte for storage. However, this is not the case in ruminants, as lipogenesis occurs within the adipocyte (Ingle *et al.* 1972,Vernon 1980, Vernon 1992). While the importance of LPL as a regulator of fat deposition is reduced in the ruminant, its activity has been suggested to supply 30-50% of the deposited fatty acids (Vernon 1992). This information suggests LPL still plays an important role in ruminant fat metabolism.

b. *de novo* Fatty Acid Synthesis

The synthesis of fatty acids requires both a source of energy to fuel the system, and a carbon source to create the fatty acid. The energy needed to fuel the system is provided in the form of NADPH, and can be derived from several pathways, including the pentose phosphate cycle, NADP-malate dehydrogenase, and the isocitrate dehydrogenase cycle. In the rat, it has been shown that the rate of lipogenesis controls the production of NADPH rather than the NADPH levels controlling lipogenesis (Vernon 1992). If this is the case in all mammals, monitoring enzymes involved in energy production may be of little use.

Figure 3 illustrates the various pathways involved in fat synthesis within the adipocyte. Acetate, glucose, lactate, pyruvate, methyl malonate, butyrate, β-hydroxybutyrate, and propionate can be used as substrates for the production of fatty acids in the ruminant, although all differ in their relative importance (Vernon 1980). In most monogastrics, glucose is the main precursor of fatty acids, entering the system at the glycolytic pathway, where its derivatives are shuttled across the mitochondrial membrane into the TCA cycle (Figure 3). From there, citrate is shuttled out of the mitochondria and transformed to acetyl CoA by isocitrate dehydrogenase. Due to microbial fermentation in the rumen, very little glucose is absorbed from the gastrointestinal tract, so most of the ruminant animal's glucose is derived from gluconeogenesis (Vernon 1980). Since the ruminant must expend a great deal of energy to produce glucose, it is in the best interest of the ruminant animal to spare glucose. Studies on ruminant animals have shown that glucose is not incorporated as efficiently as it is in other species (Vernon 1992). It was originally believed that the ruminant lacked a functional ATP-lysate-NADP malate

dehydrogenase pathway, thus preventing the animal from using glucose as a carbon source. However, studies have shown that this pathway is fully functional in bovine adipose tissue, and operates to a lesser extent in ovine tissues (Roberston *et al.* 1982). These researchers suggest that either pyruvate kinase or pyruvate dehydrogenase may be the actual points of control (Figure 3).

Acetate is the predominant carbon source for ruminant *de novo* fatty acid synthesis (Vernon 1980, Vernon 1992). Rumen fermentation produces a large amount of acetate, and it is therefore logical for the ruminant animal to use the high circulating levels of the precursor specifically for fatty acid synthesis. Vernon (1980 and 1992) reviewed several papers suggesting that acetate is incorporated into fatty acids up to 100 times faster than glucose in ruminant adipose tissues. Acetate that enters the system is converted to acetyl CoA by acetyl CoA snythetase (Figure 3), and this acetyl CoA is used to begin or elongate the growing fatty acid chain. Lactate, pyruvate, methyl malonate, butyrate, β-hydroxybuterate, propionate, and amino acids enter at various points throughout the process, but the relative importance of these precursors is thought to be minimal (Vernon 1980, Vernon 1992).

It has been shown that the activity of acetyl CoA carboxylase (ACC) is strongly correlated with the rate of fatty acid synthesis (Hardie 1989, Ingle *et al.* 1973). It is the least active of the three enzymes involved in fatty acid synthesis, catalyzing the first committed step of fatty acid synthesis (Figure 3) (Ingle *et al.* 1973). Acetyl CoA Carboxylase is an ideal candidate gene to monitor, as all precursors used in the production of fatty acids would pass through this step.

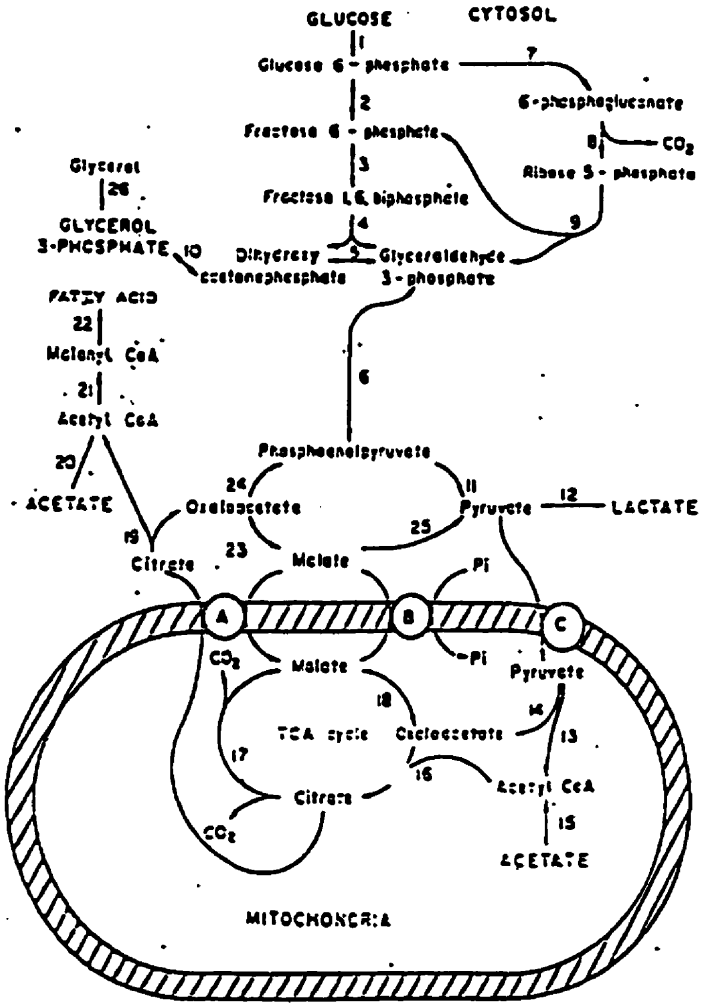

Figure 3. An overview of lipogensis within the adipocyte (Adapted from Vernon 1980).

ACC is an ATP dependent, biotin-containing enzyme that catalyzes a two step reaction resulting in the carboxylation of acetyl CoA (Voet and Voet 1990). The

mammalian ACC contains a biotin carboxylase (catalyzing reaction 1), a carboxyl transferase (catalyzing reaction 2), and a biotin binding domain (Hardie 1989). The two reactions are:

1. $ATP + HCO_3^- + Biotin\text{-}ENZYME \rightarrow ENZYME\text{-}biotin\text{-}CO_2^- + ADP + P_i$

2. $ENZYME\text{-}biotin\text{-}CO_2^- + Acetyl\text{-}CoA \rightarrow Biotin\text{-}ENZYME + Malonyl\text{-}CoA$

c. Glycerol-3-Phosphate Production

Esterification, or the attachment of 3 fatty acids to a glycerol backbone to produce a TG, must occur before fatty acids can be stored. The ruminant adipocyte has virtually no glycerol kinase activity, thus neither the glycerol released from either the uptake of circulating TG, nor the glycerol released from the cleavage of stored TG, can be used for re-esterification (Vernon 1980). The animal must use glucose, sent through the glycolytic pathway and converted to glycerol-3-phosphate by glycerol-3-phosphate dehydrogenase. Likewise, other metabolites can be used to create glycerol-3-phosphate by a reversal of the glycolytic pathway (Vernon 1980).

d. Fat Mobilization

Fatty acids are a stored source of energy, and thus are constantly being retrieved to provide fuel for metabolic activity. The TG is broken down into monoglycerol and fatty acids by the actions of two enzymes. Hormone sensitive lipase (HSL) catalyzes the first two steps in TG breakdown, hydrolyzing the 1' and 3' position fatty acids (Vernon 1992, Yeaman 1990). Studies have shown the HSL enzyme is capable of hydrolyzing the 2' fatty

acid, but at a far reduced rate (Vernon 1992,Yeaman 1990). *In vivo,* this final cleavage is performed by an enzyme known as monoglycerol lipase (Vernon 1992 and Yeaman 1990). There is no evidence to suggest that monoglycerol lipase is under complex control (Yeaman 1990), and most evidence suggests HSL is the key regulation point in lipolysis (Fredrikson *et al.* 1981).

D. Further Investigation of Potential Candidate Genes

i. Lipoprotein Lipase

a. Gene Localization and Transcript Size

The ovine LPL gene has been localized to chromosome 2 (Broad *et al.* 1995), and codes for a mRNA that shows a high percentage of sequence similarity when compared to other species (Edwards *et al.* 1993). Both human and mice express a 3.4 and 3.6 kb transcript, while bovine tissues have an additional 1.7 kb transcript (Eckel 1989, Kirchgessner *et al.* 1987). These differences are due to different polyadenylation sites (Kirchgessner *et al.* 1987). Both the 3.4 and 3.6 transcripts have a large 3' untranslated region (Ranganathan *et al.* 1995). This untranslated region plays an important role in translation, as Ranganathan *et al.* (1995) found the human 3.6 kb transcript has a higher rate of translation when compared to the 3.4 transcript.

b. Hormonal Control of LPL

In order to assess the suitability of LPL as a candidate gene, it is first important to understand the mechanism controlling its production. The regulation of LPL is complex and tissue specific. For example, lipoprotein lipase in adipose tissue is inversely regulated when compared to muscle lipoprotein lipase (Doolittle *et al.* 1990). Insulin,

catecholamines, glucocorticoids, and dietary factors have all been suggested to play a role in LPL regulation (Kirchgessner *et al.*1987).

Both *in vitro* (Fried 1990, Semenkovich *et al.* 1989, and Raynolds *et al.* 1990) and *in vivo* (Simisolo *et al.*1992) experiments revealed an increase in enzyme activity resulting from treatment with insulin. Semenkovich *et al.* (1989) found no changes in steady state mRNA levels when insulin was added to cultured 3T3-L1murine cells. Simsolo *et al.* (1992) found no changes in LPL mRNA between biopsies before and after diabetic patients were treated with insulin. In a nuclear run-on transcription assay, Raynolds *et al.* (1990) observed no increase in transcription although there was an increase in steady-state mRNA levels in the rat. This information led the authors to suggest insulin may act to stabilize the mRNA transcript in this species.

Catecholamines have also been shown to affect LPL production and activity. Isoproterenol, a β-adrenergic agonist, reduced both LPL enzyme activity and mRNA levels within two hours of addition (Raynolds *et al.*1990). Further analysis using nuclear run-on transcription showed a reduction in LPL transcription within 15 minutes of isoproterenol treatment. However, the decease in LPL mRNA levels was not fully accounted for by the drop in transcription, suggesting isoproterenol also influences transcript stability (Raynolds *et al.* 1990).

Increasing evidence suggests glucocorticoids also play a role in LPL regulation. An adrenalectomy markedly lowered adipose tissue LPL activity in obese Zucker Rats (Freedman *et al.* 1986). However, Fried and Appel (1990) found that dexamethasone alone had little effect on LPL activity in cultured human subcutaneous adipose biopsies, but the

addition of a combination of dexamethasone and insulin increased activity of LPL over 3 times as much as insulin alone. This synergism highlights the complexity of the biological system when multiple effectors are present (Fried *et al.* 1990).

c. Nutritional Control of LPL

Conflicting results exist on the exact mechanisms involved in the regulation of LPL in response to feeding. Doolittle *et al.*(1990) found an increase in rat adipose LPL mRNA levels after an overnight fast. However, Ladu *et al.* (1991) found a 60% decrease in mouse adipose LPL mRNA after a 24 hour fast. In the ruminant, LPL activity has been shown to increase in response to energy intake (Andersen *et al.* 1996). Unfortunately, mRNA levels were not measured in the previous study.

Murphy *et al.* (1993) found that certain dietary fatty acids such as fish oil and corn oil (50g/kg of feed) increased LPL mRNA levels in both the epididymal fat pad and perineal fat pads of rats. This pattern was also mirrored in the LPL activity, although it was not statistically significant. Murphy *et al.* (1993) found these changes were not accompanied by changes in insulin levels or glucose-dependent insulin trophic polypeptide (GIP), suggesting these affects are not insulin mediated. This is in conflict with the results of Monlalto and Bensadoun (1993), who found that LPL enzyme activity in adipocytes *in vitro* was effectively reduced by incubation with n-3 and n-6 fatty acid families. The differences between these two papers may be partially explained by Murphy *et al.* (1993), who suggests adipose tissues from different depots are regulated differently. In vivo, Peterson *et al.* (1990) suggests an accumulation of free fatty acids at the endothelium may actually inhibit further lipolysis by cleavage of the LPL from its anchorage. Accumulation

may have occurred in the *in vitro* experiments, causing a reduction in LPL activity.

d. Conclusions

The conflicting results seen in the LPL experiments make interpretation of the data difficult. Post-transcriptional modification may suggest the gene is not a key regulator of enzyme activity, and thus monitoring mRNA levels may be of little use. However, certain obesity-prone strains of mice, maintained on high fat diets, have been characterized as having high levels of LPL mRNA and LPL enzyme in adipose tissues when compared to other strains (Pagliassotti *et al.* 1994). Eckel (1989) cited one study that revealed a strong positive relationship between body mass index and LPL mRNA, but cautioned that most evidence suggests that increased LPL may only sustain obesity, rather than being the causative factor. Fried *et al.* (1991) suggested the obesity prone Zucker rat was due to an increase in overall protein synthesis in the adipocyte and believed this increased expression may be the result of a mutation in another gene which controls the expression of an entire subset of genes. Information has pointed directly to the LPL gene as an important regulation site in the development of obesity. Jemaa *et al.* (1995) found polymorphisms in the LPL gene that are associated with high body mass index (BMI) in obese patients.

ii. Acetyl CoA Carboxylase

a. Transcript size and Alternate Splicing

The ovine cDNA shows high sequence similarity with the previously isolated chicken (82%), rat (85%), and human (89%) cDNAs (Barber and Travers 1995). Isolated mRNA transcripts between 9.0 and 9.4 kb have been reported in sheep, with heterogeneity at both the 5' (differential splicing of primary transcript) and 3' (different polyadenylation

sites) untranslated regions producing the variability (Barber and Travers 1995). Ovine LPL is a 2345 amino acid protein with an M_r of 265 kDa (Barber and Travers 1995), which is comparable to the human protein of 2340 amino acids with a M_r of 264 kDa (Ha *et al.* 1994).

A considerable amount of research has focused on the differential splicing of ACC exons which creates 2 distinct classes of transcripts (Luo and Kim 1990, Kim *et al.* 1996, Kim and Freake 1996, Ha *et al.* 1994, Kim and Tae 1994). These classes include what Luo and Kim (1990) describe as a "housekeeping" transcript (PII) and a lipogenic form (PI), the latter regulated to a much greater extent by nutritional status and/or hormonal signals. Relative amounts of these two transcripts can't be established by northern analysis stimuli (Kim and Tae 1994, Kim *et al* 1996), and therefore may create problems when trying to quantify the response to certain. However, this differential splicing appears to be tissue specific, and PII expression is minimal when compared to PI in rat adipose tissue (Kim and Tae 1994). Similarly, Barbers and Travers (1995) found only the PI type expressed in their search for cDNAs in ovine adipose tissue. Accordingly, most studies show a high corelation between ACC mRNA levels and enzyme activity (Girard *et al.* 1994).

Ha *et al.* (1996) discovered a gene similar to ACC, coding for a 275-280 kDa protein showing a high sequence similarity with ACC. While it was first suggested to be an isoform of the ACC (Abu-Elheiga *et al.* 1995), it has since been identified as a separate gene that appears to play an important role in fatty acid oxidation, with expression primarily in heart and skeletal muscle (Ha *et al.* 1996).

b. Hormonal Control of ACC

Control at the enzyme level is accomplished by both allosteric and covalent modification (Hardie 1989). Allosteric control is exhibited by a classical feedback inhibition by long chain acyl-CoA (Hardie 1989 and Mabrouk *et al.* 1990, Vernon 1992). Likewise, citrate has been shown to dramatically activate the enzyme, which may be expected, as citrate is a precursor of cytoplasmic acetyl-CoA in most species (Hardie 1989, Davey 1986).

Covalent control of ACC has been shown by reversible phosphorylation (Hardie 1989). There is a general consensus that glucagon and epinephrine induce phosphorylation and depolymerization, increase citrate dependence, and decrease enzyme activity (Mabrouk *et al.* 1990). Recent research has shown that insulin has the opposite effects, dephosphorylating and polymerizing, increasing citrate sensitivity, and activating the enzyme (Mabrouk *et al.* 1990). While several other authors suggest dephosphorylation of ACC by insulin, Mabrouk *et al.* (1990) believes this finding was probably due to an inferior isolation technique, as ACC is rapidly phosphorylated immediately after excision of liver (Mabrouk *et al.* 1990). In any case, insulin has been shown to increase ACC activity, and this also appears to be true in ruminants as incubation of insulin with ovine adipose cells *in vitro* increases ACC activity significantly (Vernon 1992).

Mabrouk *et al.* (1990) suggests that insulin has an entirely post-transcriptional effect. However, Coupe *et al.* (1990) and Girard *et al.* (1994) found injections of insulin in diabetic rats increases both ACC enzyme activity and its mRNA levels. Insulin's effect on mRNA levels may be mediated indirectly. Iritani (1992) found that *de novo* protein

synthesis is required for insulin induced enhancement of mRNA levels, as cycloheximide (potent protein synthesis inhibitor) reduced the effect of insulin on the transcriptional rate. More recent evidence suggests that blood glucose levels, as well as insulin, influence ACC mRNA levels. A high fructose diet was also able to increase transcription rates to levels similar to those obtained by insulin treatment, causing Iritani (1992) to suggest these increases were induced by glycolytic metabolites. Fructose did not increase enzyme activity to levels seen with insulin treatment, which suggests that insulin may be required for translation or enzyme activation.

Growth hormone appears to decrease ACC mRNA levels in pigs (Lu 1994). This may also be the case in ruminants, as recombinant growth hormone decreased total ACC activity in ruminant tissue (Vernon *et al.* 1994), perhaps due to a reduction of mRNA synthesis. Thyroxine (T-3) has been shown to increase ACC mRNA (Iritani 1992, Swierczynski *et al.* 1991). This increase appears to be indirect, as protein kinase inhibitors blocked the effect of T-3 (Swierczynski *et al.* 1991).

c. Environmental Control of ACC

Most research shows ACC mRNA levels are highly correlated with ACC enzyme activity (Girard *et al.* 1994, Coupe *et al.*1990, Bai *et al.* 1986). Numerous studies have confirmed a dramatic increase in ACC mRNA levels when weaned or starved animals are fed a high carbohydrate diet (Girard *et al.* 1994, Coupe *et al.*1990, Bai *et al.* 1986, Katsurada *et al.* 1990, Hillgarnter *et al.* 1996). This increase in mRNA was directly related to an increase in transcription rate in the avian liver (Hillgarnter *et al.* 1996). Iritani (1992) suggests a direct link between feed intake and mRNA production, as an increase in

transcription paralleled the increase in the amount of food eaten in the rat. Although protein free diets were found to slightly increase levels of mRNA, Iritani (1992) found that dietary protein was needed for a substantial increase. Diurnal variation has been seen in mRNA levels, presumably due to feeding (Iritani 1992).

Other researchers have focused on the effects of specific fatty acid supplementation. Girard *et al.* (1994) found long chain polyunsaturated fatty acids appear to have an inhibitory effect on hepatic ACC mRNA levels, with almost no effect on adipose tissue mRNA. Foufelle *et al.* (1992) found that long chain fatty acids had the greatest effect on hepatic ACC, whereas medium chain fatty acids affected ACC to a lesser extent. These effects were also paralleled in adipose tissue, but on a much smaller scale. This reduction should be expected, as it would fit a classical feedback inhibition.

d. Conclusions

The close tie between ACC mRNA and enzyme activity indicates transcriptional control has a key role in the regulation of this enzyme. Differences in control points of transcription, such as the promoter region, may have drastic effects on the activity of the enzyme. This, in combination with the key role ACC plays in fat metabolism, makes this gene an obvious candidate for further investigation.

iii. Hormone Sensitive Lipase

a. Transcript size and Protein Localization

The murine HSL mRNA is estimated at 3172 nucleotides (Li *et al.* 1994), slightly smaller than the 3.3 kb transcripts reported for the human, pig and rat (Holm *et al.* 1989, Liu *et al.* 1995). Li *et al.* (1994) found high degree of sequence similarity between the

mouse transcript and the human (85%) and rat (95%), even though there was a stretch of 16 amino acids which diverged significantly among species. In adipose tissue the predominant role of HSL is that of TG breakdown (Yeaman 1990).

b. Hormonal Control of HSL

The name HSL reflects the acute control exerted on it by several different hormones (Figure 4). Lipolytic agents, such as noradrenaline released from sympathetic nerve endings, adrenaline, corticotropin and glucagon, all stimulate lipolysis (Garton *et al.* 1989). A summary of its control can be seen in Figure 4, modified from Vernon (1992).

In ruminants, the catecholamines are potent stimulators of lipolysis whereas glucagon has only a slight stimulatory effect (Vernon 1980). Both exert their effects in essentially the same way, interacting with their specific receptors in the plasma membrane, which causes the dissociation and activation of a GTP-binding protein (G_s) which in turn activates adenylate cyclase. Adenylate cyclase catalyzes the synthesis of cyclic AMP, activating the cAMP-dependent kinase (A-kinase), which in turn phosphorylates HSL at a specific serine residue that leads to activation (Stralfors and Belfrage 1983). Adenosine and prostaglandin E_2, produced in adipose tissue, activate receptors that interact with another GTP-binding protein (G_i) to reduce the cAMP concentration. This can also be accomplished by catacholamines acting via the α_2-adrenergic receptors. Therefore, the activation status of A-kinase will depend on the strengths of the various signals channeling through G_s, G_i, and phosphodiesterase.

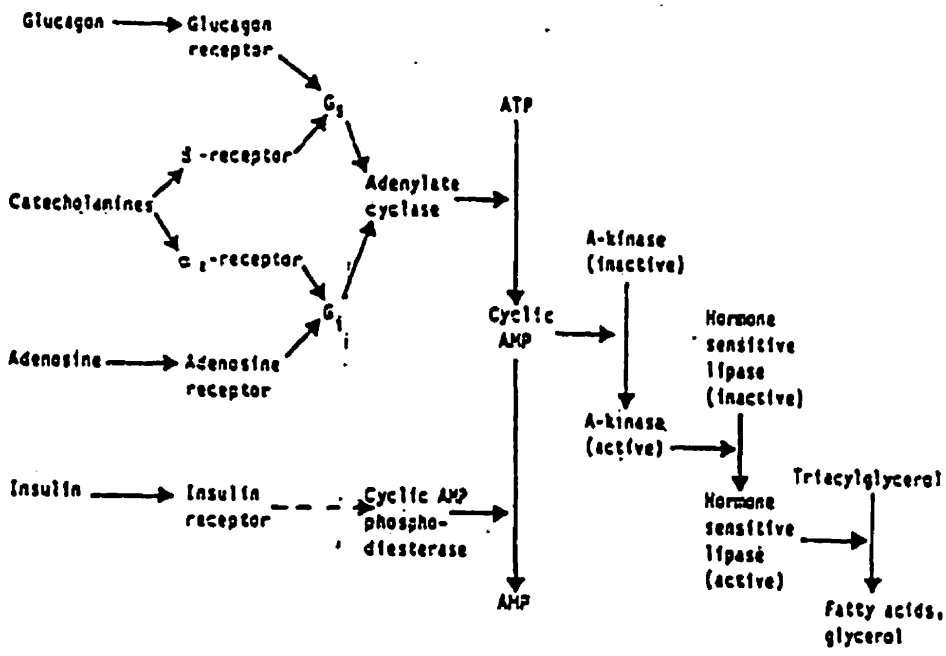

Figure 4. Pathways for control of Hormone Sensitive Lipase (Adapted from Vernon 1992).

Peptide mapping studies have revealed at least two serine residues that are phosphorylated in HSL (Yeaman 1990), a regulatory site and a basal site. Phosphorylation of the regulatory site is inhibited by insulin and increased by lipolytic hormones, and a close tie exists between lipolytic rate and phosphorylation of this site (Stralfors and Honor 1989 and Stralfors *et al.* 1984). The basal site is phosphorylated independently of lipolytic hormones, and has no direct effect on HSL activity (Yeaman 1990, Garton *et al.* 1989). However, the phosphorylation of these sites is mutually exclusive so phosphorylation of

the basal site reduces the stimulation of HSL by lipolytic hormones (Garton *et al.* 1989).

Insulin appears to inhibit HSL in two ways, the first being an activation of cyclic AMP-phosphodiesterase, which in turn decreases cyclic-AMP (Vernon 1992). Insulin also inhibits HSL through a cAMP independent mechanism, involving the dephosphorylation of HSL (Stralfors and Honnor 1989, Stralfors *et al.* 1984).

c. Other Factors Affecting HSL

One may argue that due to its acute control, regulation of HSL should occur primarily at post-translational sites. Hellstrom *et al.* (1996) found no change in HSL mRNA levels between obese and lean patients, although HSL activity was drastically reduced in obese patients. Kraemer *et al.* (1991) found no changes in HSL mRNA levels in rat adipose tissue from 3 weeks to 2 years of age. The authors further suggest that the steadiness of the levels suggest that HSL gene expression plays little if any role in the regulation of fat metabolism.

In contrast, Sztalryd *et al.* (1995) suggests the expression of the HSL gene could influence the rate of lipolysis and thus effect the amount of lipid the cell is able to accumulate. Sztalryd *et al.* (1995) transfected the 3T3-F442A cell line with the HSL gene, creating a cell line which over-expressed HSL, and showed that an over expression of HSL mRNA can prevent lipid accumulation. Immunofluorescent confocal microscopy showed transfected cells contained an abnormal distribution of HSL throughout the entire cell, while control cells showed only a faint signal in a perinuclear distribution (Sztalyrd *et al.* 1995).

On a more practical level, Sztalryd and Kraemer (1994) found prolonged fasting (72 hours) resulted in a sharp increase in HSL activity and content, which was directly related to a similar increase in mRNA levels. Liu *et al.* (1995) also found elevated levels of HSL mRNA in porcine adipose tissues after a two day fast. Wilson *et al.* (1992) found seasonal variations in HSL mRNA in the yellow-bellied marmot, with adipose tissues expressing a higher level of HSL during hibernation. Sztalryd and Kraemer (1994) suggest the long period of fasting needed to increase gene expression may show short term regulation of HSL may be due primarily to post-translational systems, or perhaps a short term mechanism (e.g. reduced LPL activity) may be sufficient to supply the excess FFA required.

d. Conclusions

The strong post-translational control exerted on the HSL enzyme may suggest the HSL gene plays little, if any, role in the regulation of the enzyme's activity. However, if post-translational control is limited to periods when acute regulation of fat metabolism is needed, regulation of the HSL gene may still provide a chronic control point. Levels of HSL expression may also prove important in the potential lipolytic cascade capacity of an animal.

iv. Adipocyte P2

a. Transcript Size, Protein Identification and Function

Several investigators have found "fat specific" proteins found only in preadipocytes and adipocytes. Among these is the aP2 gene, so named because of the striking similarity found with the mylein P2 gene (Bernlohr *et al.* 1984). The mouse aP2 gene is transcribed

into a 4.0 nucleotide mRNA, which is in turn translated into a 13,000 M_r protein. Adipocyte P2 is widely conserved in evolution and shows structural similarities with the liver fatty acid binding protein gene (Hunt *et al.* 1986), which led one researcher to suggest it's role is that of an intracellular lipid binding protein (Bernlohr *et al.* 1984). Matarese and Bernlohr (1988) believe aP2 is a lipid transporter and may also provide cytosolic protection against the detergent-like effects of fatty acids.

b. Candidate Gene Potential

Brockman *et al.* (1996) found no differences in mRNA levels of aP2 when lean and obese mice lines were compared. However, Muggli-Cockett *et al.* (1992) found significant correlations between mRNA levels of aP2 and carcass yield grade in sheep. The latter suggests that aP2 could be a useful candidate gene.

The measurement of aP2 can also provide valuable information regarding the overall activity of the fat depot. The promoter region of aP2 has significant sequence similarities to the promoter regions of other adipocyte-specific genes, suggesting a role for such sequences in the developmental regulation of fat-specific genes (Hunt *et al.* 1986). Such evidence has lead Rauscher *et al.* (1988) to suggest adipose-specific gene expression might be controlled by 'fat-specific elements', short sequences of DNA that are common to genes expressed in fat cells. Research performed on obese Zucker rats supports this theory. Dugail *et al.* (1992) showed several enzymes were over transcribed, suggesting the presence of a common trans acting factor that could trigger high expression. If this were the case, monitoring one gene from a gene group would give accurate information on an entire subset of genes controlled by that element.

v. Interaction of Candidate Genes

Acetyl CoA Carboxylase, aP2, HSL, and LPL all show great potential as possible candidate genes for the identification of economically important markers for carcass fat content. Unfortunately, single gene markers for production traits appear to be the exception rather then the rule. The underlying premise in animal breeding has always been that most traits are controlled by a large number of genes (Sellier 1994). Recent information gathered on genes involved in fat content support the multiple gene theory.

West *et al.* (1994) used Quantitative Trait Loci (QTL) analysis to link areas on chromosome 4, 9, and 15 to increased fat storage capacity in mice maintained on high-energy diets. Brockmann *et al.* 1995) found that several genes (Glycerol –3-phosphate dehydrogenase, LPL, and Insulin-like growth factor-1) appeared to affect body composition in mice. This highlights the importance of monitoring a number of genes in order to get an overall understanding of the trait to be studied.

With the potential involvement of a large number of genes, a comprehensive investigation should include the interaction of these genes and how their combination can affect carcass fat content. Often the interaction of these genes can be more descriptive than the information obtained separately on each gene. Sinnett-Smith and Wooliams (1988) found that fatness did not appear to be related quantitatively or qualitatively to either *de novo* fatty acid synthesis, uptake of exogenous fatty acids, or lipolysis, but rather to the combination of variations in all three. Shimada *et al.* (1995) found the overexpression of LPL in mouse adipose tissue caused a substantial increase in HSL expression. Similarly, Stzlyard *et al.* (1995) found an overexpression of HSL drastically decreased aP2 and

Glycerol-3-Phospho Dehydrogenase mRNA levels. Recently, Muggli-Cockett *et al.* (1992) found that the combination of LPL and aP2 mRNA levels were more effective in the estimation of carcass yield grade then if each gene was analyzed separately. For these reasons, the interaction of these genes must also be investigated.

E. Conclusions

An accurate predictor of carcass quality will have a large effect on the future of the meat animal industry. A practical selection scheme to improve carcass quality would involve several steps, with economical predictors such as visual appraisal, initially used to narrow the numbers of potential breeding stock. Reduced numbers would allow for more efficient use of more accurate and expensive techniques, such as ultrasound and finally molecular genetic analysis.

3. MATERIALS & METHODS

A. Experimental Design

i. Carcass Composition Trial

a. Source of Animals

Lambs of Rideau Arcott (R), Dorset (D), Texel (T), Texel-Dorset cross (TD) and Karakul (K) were used in this study. These breed groups were used to ensure a wide range of growth rates and carcass compositions were available to uncover any relationships between carcass traits and levels of mRNA of the genes under investigation. Complete carcass information was available from a complementary study of D, R and TD lambs.

Dorset was used because it is one of the most popular breeds in Nova Scotia. Rideau is a synthetic breed comprised of 39.9% Finnish Landrace, 19.5% Suffolk, 14.2% East Frisean with minor contributions from Shropshire, Dorset, North Country Cheviot, Leicester, Romnelet and Corriedale (Shrestha and Heary 1992). Rideau, because of the contribution of Finnish Landrace, has the tendency to accumulate a considerable proportion of its fat in the body cavity. Dorset and Rideau are maternal breeds, and have not been intensely selected for carcass traits.

Texel, one of the leanest breeds of sheep in the world, and Karakul, an unselected breed with high aptitude for fat accumulation, particularly in the tail region, were used to further expand the range of values for growth rate and carcass composition in the sample. Using animals from one breed could not have attained such range of carcass types, because there is a considerable uniformity within each breed due to selection, and the few farms keeping any particular breed in Nova Scotia. In addition, a considerable level of relatedness exists among flocks of the same breed in the region as a result of using rams from common sources and exchange of genetic material among farms. The Texel-Dorset crossbred lambs were used to have a breed leaner than Dorset but fatter than purebred Texel. This breed group also provides the opportunity of estimating heterosis for the traits studied.

Five wether lambs were used from R, D, and TD breed groups. Only three Texel lambs were used. Karakul lambs consisted of three males and two females. In both cases, availability of lambs was the limiting factor. Despite the small numbers, the Texel and Karakul lambs were included to provide a wider ranger of genetic stock for comparison.

b. Growth and Feedlot Finishing

Animals used in this study were managed according to the guidelines of the Canadian Council of Animal Care (1980). The lambs used in this study were born during March and April, had access to a creep feed (16% CP) until pastured with their dams in mid-May. In late July, animals were weaned and transferred to a feedlot. The lambs were fed hay for one week before the finishing diet of 80% whole barley and 20% a protein-mineral mix (36% crude protein) was fed. The concentrate mix contained 15% crude protein. The amount of concentrate increased, and was fed *ad libitum* three weeks after the commencement of the feedlot.

Lambs were weighed weekly, and were fasted overnight (approximately 18 hours) before slaughter. Lambs had access to water at all times. R, D and TD lambs were slaughtered when they reached the target weight of 45 kg, which is a common slaughter weight for market lambs in the region. Karakul and Texel lambs were slaughtered approximately at the same age as R and D lambs (215 days). Fasted weight was recorded within one hour of slaughter.

c. Slaughter and Carcass Measurements

The lambs were killed at provincially inspected slaughter plant by stunning followed by exsanguination. Samples from subcutaneous backfat were obtained from the right side of the carcass 5 cm down from the tail. All samples were taken within 15 minutes of animal death, placed in cryovials, snap frozen in liquid nitrogen and stored at -80°C.

Data on carcass measurement was available through another experiment. After

slaughter, body cavity fat was separated and weighed. This fat included kidney fat (perinephric and retro-peritoneal), pericardial (fat around the heart), omental (fat around the rumeno-reticulum) and mesenteric fat. Mesenteric fat included fat from the mesentery, plus associated lymphatic glands and fatty tissues pulled from the surface of the intestine. Warm carcass weight was recorded immediately after dressing and removal of offal parts, including kidneys and kidney fat.

Following chilling at approximately 5°C for about 24 h, carcasses were weighed and separated into foresaddle and hindsaddle by cutting between the 12th and 13th ribs. Backfat thickness and length and width of longissimus dorsi muscle were measured on both sides of each carcass at the 12th rib with sliding callipers. Tracings of the cross-sectional areas of the longissimus dorsi muscle at the 12th rib were used to determine area and circumference of this muscle by a Bioquant™ (System IV) image analysis system (R and M Biometrics Inc., Nashville, TN). Measurements were taken on left and right sides of each carcass in duplicate by each of two technicians. The averages of the measurements on both sides of each carcass were used for analysis.

Each of the fore- and hind-saddles was divided along the backbone, and was disjointed into nine commercial cuts (leg, loin, rack, flank, brisket, breast, shoulder, shank and neck). The tail of Karakul lambs was removed from the carcass before cutting the hindsaddle, by a round knife cut parallel to the natural curvature of the legs. This cut passed through the region of the fourth and seventh caudal vertebrae. Cuts from the left side of each carcass were tightly packed and were kept frozen for later dissection into subcutaneous fat,

intramuscular fat, bone and muscle. All muscles were individually dissected and weighed, unless they were tightly joined together with no intermuscular fat between them. The tail of Karakuls lambs was also dissected into fat, muscle and bone. Tail fat was considered as an extension of subcutaneous fat, and was added to this depot for analysis. Weight of each tissue was expressed as a percentage of sum of tissues in each cut, and in the half-carcasses

ii. Karakul Growth Trial

a. Source of Animals

The large fat depot present in the tail of the Karakul is easily accessible for repeated sampling during growth. It provides a unique model to measure simultaneously the rate of growth of the fat depot and activity of the genes involved in the regulation of fat metabolism. Five male Karakul lambs were maintained as previously described in experiment one.

b. Tissue Sampling

Biopsies were taken from the tail of each of 5 Karakul ram lambs in monthly intervals (from April to September), and another sample was taken from the tail at slaughter in October. Prior to sampling, body weight and tail length were measured. Tail circumference and thickness, at the widest point, were also recorded. A veterinarian performed all biopsies, and a local anaesthetic was used to freeze the tail. Samples were processed as previously described.

B. Laboratory Techniques

i. Extraction of Total Cellular RNA

Total cellular RNA (tcRNA) was isolated from adipose tissues using a modification of the acid guanidinium thiocyanate-phenol-chloroform method described by Chomczynski and Sacchi (1987). Whenever possible, manipulations were carried out on ice. Approximately 2g of tissue was ground to a fine powder under liquid nitrogen using a mortar and pestle, and tissues were transferred to a test tube containing 10 mL of solution D (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol). Samples were homogenized for one minute using a PT3000 Polytron Homogenizer (Brinkmann, Mississauga, Ont.) with a standard generator set at 24 000 rpm. After homogenization, 5 mL of water saturated phenol, 5 mL of chloroform:isoamyl alcohol (24:1), and 1 mL of 2 M sodium acetate (pH 4.0) were added sequentially, and tubes were subjected to vigourous agitation using a horizontal shaker (Eberbach, Ann Arbor, Mi.) for 15 minutes at 4°C. Phase separation was accomplished by a centrifugation (20 minutes, 10000*g*, 4°C) using a Beckman J2-21M/E Centrifuge and a JA-20 rotor (Mississauga, Ont.).

The aqueous phase was transferred to a new tube, and the extraction procedure was repeated twice, with the omission of the sodium acetate. RNA was precipitated by the addition of 10 mL of cold isopropanol (-20°C), followed by an incubation period of no less than 2 hours at -20°C. Samples were pelleted by centrifugation (20 minutes, 4°C, 10000*g*). The supernatant was decanted, and the pellet was resuspended in 300 µL of cold Solution

D (4°C). Samples were transferred to a microcentrifuge tube, and 300 µL cold isopropanol (-20°C) was added, followed by an incubation period of no less than 2 hours at -20°C to precipitate the sample.

Samples were again pelleted by centrifugation (10 minutes, 10 000 *g*, 4°C) using an Eppendorf 5415C micro centrifuge (Brinkman, Mississauga, Ont.), the supernate was removed, pellets were washed in cold 80% ethanol (-20°C), and dried for 3 minutes using a Savant SC110 SpeedVac (Farmingdale, NY). Pellets were resuspended in 300 µL of dH_2O. Following resuspension, 30 µL of 3M sodium acetate (pH 7.0) and 700 µL of cold 100% ethanol (-20°C) were added to each tube, and RNA was precipitated during a 2 hour incubation at -20°C. RNA was pelleted, washed, and dried as previously described, and was resuspended in 30-40 µL of cold dH_2O (4°C). An aliquot of 3 µL was retrieved for quantification, and an equal volume of cold 100% ethanol (-20°C) was added to each sample. Samples were stored at -20°C.

Sample quantity and purity was accessed by a wavelength scan from 210-310 nm using a Beckman DU-64 Spectrophotometer, and the 260/280, 260/230 ratios used to assess purity. The 260 nm reading was used to estimate the quantity of RNA present.

ii. Northern Blot Preparation

To assess RNA quality, each RNA sample was subjected to gel electrophoresis. Five micrograms of tcRNA were lyophilized and resuspended in 15 µL of gel loading buffer (2.2M formaldehyde, 20mM 3-(N-morpolino) propanesulphonic acid (MOPS), 5mM sodium acetate, 0.5mM ethylenediamine tetra acetate (EDTA), 50% formamide,

0.05% xylene cyanol, 0.05% bromophenol blue), and 1 μL of ethidium bromide (10mg/mL) was added to each sample. Samples were incubated at 65°C for 15 minutes to linearize the RNA, and then quickly placed on ice until loading onto a 1% formaldehyde/MOPS agarose gel (2.2M formaldehyde, 20mM MOPS, 5mM sodium acetate, 0.5mM EDTA). Samples were then subjected to electrophoresis for up to 8 hours at 30-40v in running buffer (20mM MOPS, 5mM sodium acetate, 0.5mM EDTA). Gels were visualized under UV light using a FBTIU-614 Fisher 312nm Variable Intensity Transilluminator (Hombe, Ont.) and samples were assessed by integrity of the 18S and 28S rRNA. Each gel also contained an RNA sample known to be intact.

Selected gels were blotted onto Hybond N™ nylon membranes (Amersham, Oakville, Ont.) using standard capillary blotting techniques (Sambrook *et al.* 1989). Gels were checked for complete transfer, blots were then marked for identification purposes and air-dried. RNA was fixed onto the membrane by UV cross-linking using the FBTIU-614 Fisher 312nm Variable Intensity Transilluminator set at 100% for 8 minutes. Processed blots were stored at 4°C until needed.

iii. Slot Blot Preparation

For the breed comparison trial, 4 blots were produced. Each blot contained a Karakul, Dorset, Texel, Texel-Dorset, and Rideau sample as well as an internal standard. For the Karakul growth trial, 10 slot blots were produced. Since the slot blot apparatus could only accommodate 6 samples, 2 blots were constructed for each of the 5 lambs. Each blot contained samples from only one animal. Blots contained either samples from periods

1,2,3,6,7, and an internal standard or samples from period 4,5,6,7 and an internal standard. The internal standard consisted of a constant sample of pooled ovine kidney fat tcRNA. This sample, common to all blots, allowed for comparisons across blots.

The Bio-dot SF slot blot apparatus (Bio-Rad, Mississauga, Ont.) was used to create slot blots for quantification. Briefly, tcRNA (28 μg) was lyophilized and resuspended in 200 μL of TE (10mM Tris-Cl, 1mM EDTA, pH 7.4), followed by the addition of 500 μL of 20XSCC (3M sodium choride, 0.3 M sodium citrate), 200 μL of formaldehyde (37% v/v), and 100 μL of dH_2O. Samples were heated to 65°C for 10 minutes to linearize the RNA, and immediately placed on ice until sample loading. Each sample was then subjected to 7 serial dilutions labeled A-H (7, 3.5, 1.75, 0.88, 0.44, 0.22, 0.11, and 0.05 μg of tcRNA) by transferring half of each subsequent sample into an equal volume (500 μL) of 10X SSC (1.5M sodium chloride, 0.15M sodium citrate). The apparatus was prepared according to the manufacturers' recommendations using Hybond™ N nylon membranes (Amersham, Oakville, Ont.). All wells were washed with 500 μL of 10XSCC before sample loading, and half (240 μL) of each serial dilution (A-H) was loaded for each sample (1-6), and all wells were then rinsed using 500 μL of 10XSCC. The apparatus was dismantled, the blot removed, and allowed to air dry. The blotting procedure was repeated using the remaining half (240 μL) of each serial dilution (A-H) for each sample (1-6). Thus, duplicate blots containing 7, 3.5, 1.75, 0.88, 0.44, 0.22, 0.11, and 0.05 μg of tcRNA were produced for 6 RNA samples.

RNA was fixed onto the membrane by an UV cross-linking using the FBTIU-614

Fisher 312nm Variable Intensity Transilluminator set at 100% for 8 minutes. Processed blots were stored at 4°C until needed.

iv. Source of Probes

The human ß-actin (BA) cDNA (1.8Kb, Genbank DNA Accession #M10278, Genbank mRNA Accession #X00351) was purchased from Clonetech (Palo Alto, CA). Since BA codes for a cytoskeleton protein, it has long suggested that mRNA levels should be constant in each cell. Therefore, BA provides an internal standard to ensure equal amounts of tcRNA have been loaded for each sample analyzed. The mouse aP2 probe was a 400 bp partial cDNA (Genbank Accession #M13261-M13264) cloned into the *Pst* I site of pBR322 and was kindly provided by Dr. B.M. Speigelman (Dana-Farber Cancer Institute, Boston, MA) through Dr. N.E. Cockett (Utah State University). The mouse HSL probe, a 1.7kb cDNA (Genbank #U08188) kindly provided by Dr. K. Reue (VA Medical Center, University of California, Los Angeles), was cloned into the *EcoR*I-*Hind* III sites of pGEM2. The ovine LPL probe was a 1.7kb cDNA (Genbank #X68308) cloned into the *EcoR*I site of pBluescript kindly provided by Dr. W.Edwards (Dept. of Biochemistry, University of Wales). The ovine ACC probe, a 9kb cDNA (Genbank #X80045) cloned into the *Not*I site of pGEM-5zf+, was kindly provided by Dr. M.C. Barber and Dr. M.T. Travers (Hanah Research Institute, Scotland).

v. Probe Preparation

All DNA modifying enzymes and restriction enzymes were purchased from New England Biolabs (Mississauga, Ont). For convenience, the aP2 probe was removed from

the pBR322 plasmid and cloned into the pBluescript KS+ from Promega (Hornbe, Ont.) using a modification of standard ligation procedures (Sambrook *et al.* 1989) in which the aP2 fragment was phosphorylated prior to ligation to increase recombination. Ligation products were used to transform Promega competent HB101 cells.

Briefly, one fifth of each ligation reaction and 40 μL of competent cells were placed on ice for half an hour, followed by a 4 minute incubation at 37°C. Four hundred microlitres of LB broth (2% bacto-tryptone, 1% yeast extract, 2% sodium chloride) was added to each tube, followed by an additional 45 minute incubation at 37°C. Transformations were then plated out on LB-ampicillin plates (2% bacto-tryptone, 1% yeast extract, 2% sodium chloride, 4% agar, with ampicillin added to a concentration of 50 $\mu g \cdot mL^{-1}$), and incubated at 37°C for 14 hours. Colonies were picked and cultured in LB-ampicillin broth, and plasmid DNA isolated using a modification of Sambrook *et al.* (1989) alkaline lysis extraction technique, substituting 2M sodium acetate for 2M ammonium acetate. Isolated plasmid was then quantified using the Beckman DU-64 Spectrophotometer.

For aP2, the plasmid (1ng) was than added to a 100 μL PCR reaction mixture [20 pmol each of Pharmacia M13 Universal Forward and Reverse Primers, 5 units of Boehringer Mannheim Taq Polymerase (Boehringer Mannheim, Laval, PQ), 192 μM of Promega dNTPs, and 1x Boeringer Mannheim PCR reaction buffer]. The aP2 probe was amplified in a MJ Research Inc. (Watertown, Mass.) PCR-100™ Thermocylcer using the 3 step program (94°C for 1 minute, followed by 30 cycles of 92°C for 30 seconds, 60°C

for 30 seconds, and 75°C for 1 minute 30 seconds; the last step included a five minute incubation at 75°C). PCR product was cleaned using chloroform: phenol (50:50) extraction, followed by ethanol precipitation, and resuspension in 20 μL of 1xTE. Probes were excised from 20 ug of plasmid using a 100ul digestion mixture [(LPL- 20 units of EcoRI, 50mM NaCl, 100mM Tris-HCl, 10mM $MgCl_2$, 0.025% Triton X-100). (HSL- 20 units of EcoRI and HindIII, 50mM NaCl, 100mM Tris-HCl, 10mM $MgCl_2$, 0.025% Triton X-100), and (ACC- 20 units of XhoI, 50mM NaCl, 50mM Tris-HCl, 10mM $MgCl_2$, 1mM DTT, supplemented with 25 μg/mL of bovine serum albumin)] incubated at 37°C overnight.

A one-tenth volume of gel loading buffer (50% glycerol, 20mM Tris-HCl, 10mM EDTA, 0.02% bromophenol blue, 0.02% xylene cyanol) was added to the digested DNA, and the samples were subjected to gel electrophoresis in a 1% low melting point agarose gel (FMC, Rockland, Me.) using a 1xTBE running buffer (45mM Tris-Borate, 1mM EDTA) to separate DNA fragments. The resulting gel was stained for half an hour in an ethidium bromide solution ($1\mu g \cdot mL^{-1}$), and the DNA of interest were excised from the gel under UV light. The gel slice was placed in a microcentrifuge tube, and a 20mM Tris-HCL, 1mM EDTA solution (2 times the volume of the agarose gel slice) was added, followed by incubation at 65°C for five minutes to melt the agarose. An equal volume of buffered phenol (pH>7.4, prewarmed to 65°C) was added, the solution was vigorously mixed for one minute, and was then subjected to centrifugation at 14,000 rpm. The aqueous layer was then extracted once with an equal volume of chloroform: phenol (50:50), and then once

with an equal volume of chloroform. A one tenth volume of sodium acetate (2M, pH 5.2) and two volumes of cold 100% ethanol (-20°C) were added to precipitate the DNA. The DNA was pelleted by centrifugation (10 minutes, 14 000 rpm), washed in 500μL of cold 80% ethanol (-20°C), and allowed to air dry for 30 minutes.

The concentration of probe was measured using Sambrook *et al.* (1989) protocol for estimating the concentration of small amounts of nucleic acids. Briefly, a 1% agarose gel slab containing ethidium bromide (1μl mL^{-1}) was cast. Two microlitres of solution containing 1, 2, 5, 10, and 20 ng of standard DNA was dotted onto the agarose. One microlitre of the DNA to be quantified was also dotted onto the agarose. The solution was allowed to permeate the gel for 1 hour. The gel was visualized under UV light, and the standards were used to estimate sample concentration.

DNA probes (25ng) were labeled using a Multiprime DNA labeling kit (Amersham, Oakville, Ont.) and [α^{32}P] dATP (ICN, Aurora, Ont.). Unincorporated nucleotides were removed from the solution using a ProbeQuant G-50 MicroColumn (Pharmacia, Bai d'Urfe, Quebec). Specific activity of purified probe was measured using a Beckman LS3801 Liquid Scintillation System (Mississauga, Ont.) and was routinely over $1x10^9$ cpm per μg of DNA.

vi. Hybridization

Blots were hybridized to probes in disposable 50mL Falcon tubes®, placed in hybridization tubes. If multiple blots were used, nylon mesh was used to separate each blot. Blots were prehybridized in 5mL of hybridization solution [5xSSPE (0.75M sodium

chloride, 0.075M monobasic sodium phosphate, 5mM EDTA), 5x Denhart's solution (0.1% polyvinylpyrrolidone, 0.1% bovine serum albumin, 0.1% Ficoll® 400), 0.5% sodium dodecyl sulfate, 50% formamide] for no less than 2 hours set at 3 rpm and 42°C in a Robbins Scientific Model 400 Hybridization Incubator (Sunnyvale, Ca.). Labeled probe (12.5 ng) was added to each tube and hybridization carried out for no less then 12 hours.

Blots were washed 2x 10 minutes in Wash buffer I [2xSSPE (0.3M sodium chloride, 30 mM monobasic sodium phosphate, 5mM EDTA), 0.1% SDS] in the hybridization incubator (10rpm, 42°C), followed by 3 washes for 15 minutes in wash buffer II [1xSSPE (0.15M sodium chloride, 15mM monobasic sodium phosphate, 2.5 mM EDTA, 0.1%SDS, preheated to 55°C], followed by a more stringent wash which varied according to probe. HSL blots were subjected to an additional half hour wash in Wash buffer II (preheated to 55°C), while blots probed with aP2, BA, LPL, and ACC were washed 2x10 minutes with wash buffer III [0.2xSSPE (30mM sodium chloride, 3mM monobasic sodium phosphate, 0.5mM EDTA), 0.1%SDS, preheated to 55°C].

Washed membranes were retrieved from tubes, and excess liquid blotted off. Membranes were then sandwiched between 2 pieces of cling wrap, labeled, and placed in cassettes containing DuPont Cronex® III-Plus XK intensifying screens. Kodak OMAT XAR film (Rochester, NY) was exposed to membranes for up to 20hours at -80°C. The resulting autoradiographs were labeled and stored until densitometric analysis.

If membranes were reused, they were first stripped of radioactive probe using a 0.1% SDS

solution which was heated to boiling and poured over blots and allowed to cool to room temperature with gentle agitation. Stripped blots were checked for residual radioactivity before use.

C. Quantification of Autoradiograph

i. Estimation of Optical Density Values

Resulting autoradiographs were scanned using an XRS omni media scanner 12cx (Torrance, Ca), and Optical densities (OD) were determined using BioImage Densitometric Software (Ann Arbor, Mi.). The reason for applying eight levels of tcRNA onto a blot is to find the most accurate estimate of the band intensity (OD) at a specific level of tcRNA loaded to a slot, regardless of differences in exposure time. The OD values are plotted against the amount of tcRNA loaded onto the slot, and the technician then decides which values are within the linear range, and a standard curve can be generated using these values. The standard curve equation can then be used to compute an OD value for a given amount of tcRNA. However, this technique is fairly subjective, and therefore variation may be introduced due to the technicians subjective decisions regarding which points fall within the linear range.

The relationship between the amounts of tcRNA applied to each slot (tcRNA load) and the OD values followed an S-shaped curve, i.e. a small increase in OD at low levels of tcRNA load, followed by a rather sharp increase over the medium levels, and a reduction in the rate of increase in OD at high levels of tcRNA load. This relationship was observed because bands at low levels of tcRNA load were rather faint, and differences between their

intensities (OD) fell below the precision of the scanner and could not be accurately assessed. Sometimes the band at the lowest level of tcRNA load was so faint that it could not be detected at all (zero value). As the amount of tcRNA load increased, the bands' intensity increased, and there was a level of band intensity beyond which any further increase in tcRNA load was not manifested in a corresponding increase in the band's intensity. The shape of the curve varied depending on the amount of mRNA present in a sample and the exposure time. The bands at the low levels of tcRNA load may be faint if mRNA in a sample is low (although tcRNA was the same in all the samples at each level) or exposure time was not sufficiently long. In such cases, one or two bands at the left side of the curve (low level of tcRNA load) were faint or missing, and the curve reached a plateau only at very high levels of tcRNA load. When a sample contained high amounts of mRNA and/or exposure time was long, the bands at the low levels of tcRNA load were intense, and the curve plateaued at a lower level of tcRNA load.

Several regression equations were tested to linearize the relationships between the amounts of tcRNA loaded on each blot and the OD values. The results indicated that OD values have a linear relationship with the logarithm of tcRNA applied to each blot. The quadratic terms used in the models were not significant in any case, and R^2 values were very high, mostly larger than 0.90. The OD values for each sample were then computed at log (tcRNA load)=0, corresponding to 1.0 μg of tcRNA loaded onto a blot. The same procedure was used in computing the OD of the β-actin.

ii. Adjustments Among Blots

Due to the large sample size, more than one blot was constructed. Since numerous blots were necessary, comparisons of samples on different blots were necessary. To ensure that comparisons could be made across blots, every blot used in the present study contained a sample of tcRNA from kidney fat that was pooled prior to the start of the experiment. Therefore, all blots analyzed contained a common sample. Blots were then linearly adjusted using this internal standard.

iii. Adjustments Using β-actin

The values of OD obtained in this manner are measures of mRNA of the gene of interest per 1.0 μg of tcRNA. Although tcRNA is strongly correlated with the number of cells in the sample, those samples containing larger fat cells have less tcRNA because fat comprises the bulk of a large cell's volume. Extracting tcRNA from a specific amount of tissue, and expressing mRNA per unit weight of the tissue is not practical because it is impossible to completely extract RNA from a sample, and some amount of RNA is always lost during the extraction process. A practical method of adjusting mRNA levels for the number of cells is to use β-actin mRNA as a reference. β-actin is a cytoskeletal protein found in all cells, and its amounts are believed to be relatively constant from cell to cell (Sambrook *et al.* 1989). Therefore, relating mRNA levels of a specific gene to β-actin provides a quick and efficient method for standardization of samples according to cell numbers. Unfortunately, research has now shown that these "housekeeping genes" may also be subject to variability as well (Spankis 1993). Therefore, data was analyzed both with and without β-

actin standardization.

D. Statistical Analysis

i. Carcass Composition Trial

Differences between breed groups for growth parameters, slaughter traits and proportion of carcass tissues were analyzed using a one-way analysis of variance. Means were compared using Scheffe's method when the F value was significant ($P<0.05$). These analyses were performed to demonstrate the contribution of various breed groups to the observed variation. The breed group means may not be accurate estimates for these breeds due to the small number of observations, and the disregarding of factors such as sex, type of birth and age of dam, which have been shown to influence growth rate and carcass composition. Any significant difference would indicate the presence of a large difference among breed groups that can even be detected using a small number of animals.

One-way analysis of variance was used to compare breed groups for the OD of the four genes, OD of β-actin, and the ratio of the gene's OD over β-actin's OD. Growth rate and carcass traits were regressed on the linear and quadratic terms of mRNA levels. A separate analysis was performed for each gene, once using the actual mRNA and then the mRNA levels adjusted for β-actin. In order to identify the genes which best describes the variation in carcass trait, linear and quadratic terms of the four genes, and the product of all possible combination of the genes were used in a stepwise regression procedure.

All the analyses were performed using the SAS computer program (Statistical Analysis System 1996). Means were compared using the Scheffe's method when F values

in the analysis of variance were significant ($P<0.05$) or approached significance ($P<0.10$).

ii. Karakul Growth Trial

Data on monthly weights, daily gains, tail measurements, and mRNA levels of the genes were analysed using the following model:

$$y_{ij}=\mu+A_i+P_j+e_{ij}$$

where μ is the overall mean, A_i is the effect of the i^{th} lamb, P_j is the effect of the j^{th} period, and e_{ij} is the random error. The effect of individual lambs was included in the model because several measurements were taken on each laboratory (repeated measurements). Means were compared using the PDIFF option of the GLM procedure of SAS.

The relationships between tail measurements and mRNA levels were computed by adding the mRNA of each gene as a covariate to the above model, i.e.,

$$y_{ij}=\mu+A_i+P_j+b(x_{ij}-x)+ e_{ij}$$

where b is the regression coefficient of weight, gain or tail measurements on mRNA of each gene, x_{ij} is the level of mRNA of the i^{th} lamb in the j^{th} period, and x is the mean level of mRNA of each gene. Regression coefficients calculated in this fashion are the within group (lamb and period) regressions. They are computed as:

$$b=SS_{xy}\text{ (error)}/SS_x\text{ (error)}$$

where SS_{xy} is sum of cross-products of dependent (Y) and independent (X) values after the

effects of lamb and period are removed (error sum of cross-products), and SSx is the independent variable's error sum of squares. The proportion of variation in dependent variables that was explained by the independent variable (mRNA levels) was computed by subtracting the R^2 of model 2 from R^2 of model 1. The ratio of mRNA of the genes and their cross-products were also analysed following the same procedures.

E. Confirmation Using Northern Blotting

Northern blots revealed hybridization conditions for all genes could not always be optimized to create a sharp band with low background. To test the accuracy of slot blots, a northern blot was constructed using all seven mRNA samples extracted for the growth trial from one individual lamb. This blot was then probed with both LPL and ACC, and β-actin. Data was compared to results obtained from slot blot analysis.

4. RESULTS

A. Carcass Composition Trial

i. Carcass Measurements

Descriptive statistics for carcass tissue composition, backfat thickness, and dimensions of longissimus dorsi muscle for the entire sample and for each individual breed group are shown in Tables 1 and 2. Percentages of subcutaneous fat in the Texel lambs were the smallest among the breed groups, ranging from 6.8% to 9.9%, and did not overlap with the values in Rideau, Dorset and Karakul (10.4% to 23.6%, Table 1). Intermuscular fat in the Texel (8.8% to 11.4%) and Karakul lambs (7.4% to 12.9%) just slightly overlapped with those in Rideau and Dorset lambs (12.3% to 20.5%). The Texel lambs had the lowest percentages of total carcass fat (15.6% to 21.3%) and the highest

Table 1. Carcass Characteristics of Texel, Rideau, Dorset, Karakul, and Crossbred lambs[1].

Measurement	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul
Subcutaneous fat, %						
Minimum	6.8	6.8	10.4	11.9	8.4	14.3
Maximum	23.6	9.9	17.2	20.6	15.2	23.6
CV	29.6	20.7	19.1	21.7	14.3	18.6
Intermuscular fat, %						
Minimum	7.4	8.8	12.9	12.3	10.2	7.4
Maximum	20.5	11.4	20.5	19.3	15.3	12.9
CV	25.3	14.1	17.8	18.3	14.3	20.6
Total carcass fat, %						
Minimum	15.6	15.6	23.3	26.1	18.6	21.8
Maximum	37.8	21.3	36.4	37.8	30.4	36.6
CV	23.4	17.1	17.3	18.8	18.1	18.6
Muscle, %						
Minimum	45.6	61.4	46.2	45.6	50.0	46.7
Maximum	67.8	67.8	54.6	53.7	59.3	54.6
CV	10.9	5.0	6.1	7.6	6.2	6.1
Bone, %						
Minimum	14.0	14.4	14.0	14.2	14.6	14.0
Maximum	20.2	17.0	18.1	18.3	18.7	20.2
CV	10.2	8.4	9.4	11.0	9.4	12.8
Muscle:Subcutaneous fat						
Minimum	1.21	2.88	1.26	1.21	1.64	1.28
Maximum	4.10	4.10	2.35	2.02	3.18	2.51
CV	38.8	18.8	24.9	24.9	26.2	24.9
Muscle:Intermuscular fat						
Minimum	1.98	6.18	2.83	2.22	3.30	1.98
Maximum	9.31	9.31	5.26	4.44	7.06	3.81
CV	47.5	21.6	27.0	28.0	31.4	22.8
Muscle:Total carcass fat						
Minimum	2.25	5.41	2.25	2.36	3.27	3.61
Maximum	7.41	7.41	4.24	4.35	5.79	7.33
CV	35.2	16.5	24.2	24.4	21.8	29.7

1-Weight of each tissue as a percentage of all tissues in half a carcass. Percentage of miscellaneous tissues is not shown. Tissues in the tail of Karakul lambs are included. Tail fat was added to the subcutaneous fat.

Table 2. Range values and coefficient of variation of backfat thickness and dimensions of longissimus dorsi (LD) muscle.

Measurement	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul
Backfat thickness, mm						
Minimum	1.0	1.0	1.9	2.2	1.5	1.3
Maximum	9.2	3.2	7.7	9.2	7.3	4.4
CV	62.1	56.4	56.9	61.9	57.4	47.3
LD muscle width, cm						
Minimum	4.4	6.2	5.1	5.5	5.6	4.4
Maximum	6.6	6.6	5.8	6.1	6.1	5.1
CV	10.4	3.1	7.0	4.4	3.9	5.6
LD muscle depth, cm						
Minimum	1.8	3.1	2.6	2.7	3.2	1.8
Maximum	3.7	3.3	3.3	3.1	3.7	2.8
CV	15.6	3.6	10.9	6.9	5.6	16.9
LD muscle area, cm^2						
Minimum	9.1	16.4	10.7	11.2	13.7	9.1
Maximum	20.3	20.3	14.1	13.8	16.5	12.8
CV	21.2	11.1	11.1	9.0	8.1	15.6
LD muscle circumference., cm						
Minimum	12.8	16.4	13.9	14.4	15.5	12.8
Maximum	19.0	19.0	15.6	15.8	17.0	15.2
CV	9.4	7.5	5.4	4.1	3.8	6.9

percentages of carcass muscle (61.4% to 67.8%), which did not overlap with those in Rideau, Dorset and Karakul. These values show the importance of Texel in increasing the range of carcass tissue composition in the entire sample. Variations among and within breed groups for percentage of bone were small.

Backfat thickness showed the largest coefficient of variation (47.3 in Karakul to 61.9 in Dorset lambs, Table 2) amongst the traits studied, as a result of the large degree of variability among the lambs within each breed group. This variability did not entirely manifest itself in the percentage of subcutaneous fat, where coefficients of variation ranged from 14.3 in Karakul to 21.7 in Dorset (Table 1). The smaller CV of percentage of subcutaneous fat than backfat thickness is very likely due to the fact that fatter lambs with thicker backfat tend to be heavier, and part of the variation in backfat thickness dissipated when percentage of tissues was used. Ratios of carcass tissues were the second most variable measures of carcass composition, with coefficients of variation ranging from 18.8 to 31.4.

As expected, coefficients of variation were larger in the entire data set than those in each breed for all the traits studied. The increase in coefficient of variation in the entire data set over those in individual breeds was particularly evident in the case of ratio of carcass tissues.

Means of growth, slaughter characteristics and carcass composition of the breed groups are shown in Table 3. Averages of slaughter age ranged between 193 days in Texel-Dorset to 220 days in Karakul lambs, and were not different among breed groups (Table 3). The Karakul lambs had a lighter fasted slaughter weight and chilled carcass weight ($P<0.01$) than the other breed groups, which were not different for these

Table 3. Means of growth and carcass characteristics by breed group.

Measurement	Texel	Rideau	Dorset	Texel-Dorset	Karakul	Pr
Slaughter weight[3], kg	47.3 a	41.6 a	42.2 a	43.0 a	31.7 b	0.001
Slaughter age, d	215	216	213	193	220	0.39
Gain per day of age, g	208 a	188 ab	183 ab	213 a	133 b	0.005
Chilled carcass, kg	24.3 a	20.1 a	20.6 a	21.2 a	15.4 b	0.001
Body cavity fat, %[1]	2.31 a	5.80 b	3.99 ab	3.38 ab	3.50 ab	0.028
Carcass tissue, %[2]						
Subcutaneous fat (SCF)	8.0 a	14.2 ab	16.0 b	12.2ab	18.2 b	0.002
Intermuscular fat (IMF)	9.8 a	16.6 c	15.4 bc	13.1 abc	10.6 ab	0.002
Total carcass fat (TCF)	17.8 a	30.8 b	31.9 b	25.3 ab	28.8 ab	0.013
Muscle	64.2 a	50.3 b	49.5 b	54.9 b	50.3 b	0.000
Bone	15.6	16.1	16.5	16.8	17.4	0.68
Ratio of carcass tissues						
Muscle:TCF	3.68 a	1.69 b	1.64 b	2.26 b	1.82 b	0.000
Muscle:SCF	8.23 a	3.69 b	3.25 b	4.78 b	2.86 b	0.000
Muscle:IMF	6.68 a	3.13 b	3.33 b	4.29 b	4.99 ab	0.001
Backfat thickness, mm	2.0	4.2	4.8	4.3	2.5	0.32
Longissimus dorsi muscle						
Width, cm	6.47 a	5.50 b	5.71 b	5.93ab	4.81 c	0.000
Depth, cm	3.21ab	2.80bc	2.92ab	3.39 a	2.33 c	0.000
Area, cm^2	18.1 a	12.4bc	12.9bc	15.3ab	10.3 c	0.000
Circumference, cm	17.6 a	14.9bc	15.1bc	16.2ab	13.7 c	0.000

1-Kidney, pericardial, omental and mesenteric fat as a percentage of fasted slaughter weight.
2-Weight of each tissue as a percentage of all tissues in half a carcass. Percentage of miscellaneous tissues is not shown. Tissues in the tail of Karakul lambs are included. Tail fat was added to the subcutaneous fat.
3- a,b,c denote significant differences among means within the same row.

measurements. Gain per day of age in Texel-Dorset (213 gd^{-1}) and Texel lambs (208 gd^{-1}) was significantly higher than that of Karakul lambs (133 gd^{-1}). The Rideau (188 gd^{-1}) and Dorset breeds (183 gd^{-1}) had intermediate growth rates.

The breed groups used in this study showed notable differences ($P<0.01$) in the pattern of fat deposition. Rideau lambs stored a significantly higher amount of fat in the body cavity (5.80% of fasted slaughter weight) and as intermuscular fat (16.3% of cold carcass weight) than T lambs (2.31% and 9.8%, respectively). Other breed groups had intermediate values for body cavity fat (3.38% in Texel-Dorset to 3.99% in Dorset lambs) and intermuscular fat (10.6% in Karakul to 15.4% in Dorset). Karakul lambs, on the other hand, deposited a high proportion of their carcass fat subcutaneously (18.2%), most in the tail. Dorset lambs also had a high percentage of subcutaneous fat (16.0%), which was not different from that in Karakul lambs. The lack of a significant difference among the breed groups for backfat thickness, despite the large variation among them, is caused by large differences among lambs within each breed group, as shown by the large coefficients of variation.

Texel lambs had the highest percentage of muscle, muscle to total carcass fat ratio, and muscle to subcutaneous fat ratio amongst all breed groups ($P<0.01$). Muscle to intermuscular fat was the highest in Texel and the lowest in Rideau, Dorset and Texel-Dorset lambs, while Karakul lambs were intermediate. Dimensions of longissimus muscle were the smallest in K and the largest in Texel and Texel-Dorset lambs ($P<0.01$). The growth and carcass traits in Texel-Dorset lambs were intermediate between the Texel and Dorset breeds that made up the cross. Percentage of carcass muscle was the only measurement that was significantly different between Texel-Dorset and the Texel

Table 4. Descriptive statistics of mRNA of the genes (unadjusted) by breed group.[1]

Gene	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul	Prob.[2] (MSE)
ACC							
Mean	0.508	0.488	0.547	0.445	0.510	0.544	0.75
SD	0.129	0.092	0.131	0.157	0.060	0.181	(.018)
Min.	0.172	0.391	0.409	0.172	0.406	0.234	
Max.	0.740	0.574	0.740	0.572	0.560	0.685	
CV	25.3	18.9	24.0	35.4	11.8	33.2	
LPL[3]							
Mean	0.478	0.187 a	0.380 ab	0.595 b	0.596 b	0.515 ab	.026
SD	0.214	0.140	0.175	0.148	0.179	0.217	(.031)
Min.	0.087	0.087	0.200	0.410	0.320	0.233	
Max.	0.816	0.348	0.602	0.758	0.816	0.771	
CV	44.9	74.7	46.1	24.8	30.0	42.0	
HSL							
Mean	0.916	1.006	0.871	0.947	0.907	0.884	.28
SD	0.094	0.121	0.093	0.078	0.053	0.110	(.008)
Min.	0.737	0.870	0.764	0.817	0.817	0.737	
Max.	1.102	1.102	0.967	1.026	0.948	0.979	
CV	10.2	12.0	10.6	8.2	5.8	12.5	
aP2							
Mean	0.601	0.578	0.589	0.628	0.616	0.584	.84
SD	0.072	0.077	0.078	0.086	0.057	0.083	(.006)
Min.	0.480	0.491	0.487	0.555	0.525	0.480	
Max.	0.762	0.635	0.677	0.762	0.667	0.712	
CV	12.0	13.3	13.2	13.8	9.3	14.2	

1- Values used in this table represent arbitrary OD units

2-Probability of differences between breed groups. Man square error is shown in bracket.

3- a,b,c denote significant differences among means within the same row.

Table 5. Descriptive statistics of mRNA of the genes adjusted for β-actin by breed group.[1]

Gene	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul	Prob.[2] (SEM)
ACC							
Mean	0.554	0.541	0.613	0.467	0.557	0.586	0.63
SD	0.148	0.076	0.157	0.161	0.078	0.213	(.023)
Min.	0.185	0.493	0.487	0.185	0.445	0.270	
Max.	0.876	0.629	0.876	0.576	0.667	0.818	
CV	26.7	14.0	25.6	34.4	14.1	36.3	
LPL							
Mean	1.590	0.703	1.351	1.941	1.901	1.698	0.15
SD	0.765	0.701	0.744	0.634	0.483	0.915	(.504)
Min.	0.205	0.205	0.722	1.259	1.464	0.746	
Max.	3.081	1.504	2.635	2.886	2.652	3.081	
CV	48.2	99.7	55.0	32.7	25.4	53.9	
HSL							
Mean	1.670	1.969	1.517	1.752	1.490	1.741	0.67
SD	0.483	0.491	0.523	0.482	0.320	0.636	(.252)
Min.	0.903	1.405	0.903	1.180	1.147	0.919	
Max.	2.512	2.299	2.186	2.310	1.857	2.512	
CV	28.9	24.9	34.5	27.5	21.5	36.5	
aP2							
Mean	1.174	1.351	1.099	1.157	1.087	1.246	0.73
SD	0.290	0.282	.323	0.243	0.104	0.448	(.092)
Min.	0.716	1.075	.739	0.897	0.980	0.716	
Max.	1.945	1.640	1.568	1.407	1.258	1.945	
CV	24.7	20.9	29.4	21.0	9.6	35.9	

1- Values used in this table represent the ratio of the specific mRNA to β-actin mRNA
2-Probability of differences between breed groups. Mean square error is shown in brackets

breed. Percentages of subcutaneous fat, intermuscular fat, total carcass fat, muscle and bone showed 1.7%, 3.9%, 1.8%, -3.4% and 4.7% heterosis, respectively.

ii. Breed Differences in mRNA Levels

The ACC, aP2 and HSL mRNA levels were not different across breeds for either unadjusted (Table 4) or ß-actin-adjusted values (Table 5). However, LPL mRNA levels showed significant differences among breeds, with Texel lambs showing a lower value (0.478, arbitrary units) when compared to Dorset (0.595) and Texel-Dorset (0.596). The Rideau and Karakul breeds had intermediate values. The significant differences across breeds were removed when data was adjusted using β-actin mRNA levels. In the case of all the genes, probability levels and mean square errors increased when adjusted values were used compared with unadjusted values.

Ratios of the ACC, aP2, HSL, and LPL mRNA levels revealed several significant differences across breed groups. The Dorset breed had a significantly higher LPL/ACC value (1.487) compared with Texel (0.429) and Rideau (0.681) (Table 6). When ß-actin adjusted values were used, only the Dorset breed had a significantly higher (4.472) LPL/ACC ratio than Texel (1.365) (Table 7). Breed differences for LPL/HSL ratio also approached significance (P=0.058) when unadjusted mRNA levels were compared (Table 6). Texel had the smallest LPL/HSL ratio of all breeds (0.681 to 0.983. For ß-actin adjusted ratios, the Texel breed showed a significantly lower LPL/aP2 ratio (0.469) when compare to both the Dorset (1.706) and Texel-Dorset (1.753) breed.

The Texel-Dorset cross showed 9.3%, 52.4%, -7.1% and 2.2% percent heterosis for the unadjusted ACC, LPL, HSL and aP2 mRNA, respectively. The corresponding values for the adjusted mRNA were 10.5%, 43.8%, -19.9% and -13.3%.

Table 6.Descriptive statistics of the ratio of mRNA of the genes (unadjusted) by breed group.

Gene	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul	Prob. (MSE)
LPL/ACC							
Mean	0.985	0.429a	0.681a	1.487b	1.147ab	0.958ab	0.004
SD	0.479	0.399	0.221	0.565	0.243	0.244	(0.1260)
Min.	0.174	0.174	0.432	0.975	0.788	0.562	
Max.	2.39	0.889	0.870	2.390	1.456	1.186	
CV	48.6	93.0	32.4	38.0	21.2	25.5	
LPL/HSL							
Mean	0.534	0.183	0.451	0.636	0.650	0.609	0.058
SD	0.256	0.129	0.242	0.174	0.169	0.312	(.0495)
Min.	0.100	0.100	0.237	0.400	0.392	0.251	
Max.	0.968	0.332	0.743	0.785	0.860	0.968	
CV	48.0	70.8	53.7	27.4	25.9	51.2	
LPL/aP2							
Mean	0.813	0.351	0.681	0.983	0.956	0.909	0.14
SD	0.392	0.311	0.390	0.337	0.241	0.466	(.1311)
Min.	0.142	0.142	0.320	0.538	0.609	0.405	
Max.	1.608	0.708	1.139	1.345	1.291	1.608	
CV	48.2	88.6	57.3	34.3	21.3	51.3	
ACC/HSL							
Mean	0.566	0.489	0.641	0.481	0.561	0.627	0.52
SD	0.172	0.104	0.199	0.194	0.039	0.229	(.0304)
Min.	0.167	0.373	0.423	0.167	0.497	0.252	
Max.	0.913	0.573	0.913	0.701	0.591	0.860	
CV	30.3	21.2	31.1	40.4	6.9	36.5	
ACC/aP2							
Mean	0.866	0.840	0.961	0.741	0.828	0.952	0.71
SD	0.269	0.057	0.337	0.306	0.056	0.378	(.0789)
Min.	0.225	0.797	0.604	0.225	0.773	0.407	
Max.	1.429	0.905	1.399	1.031	0.888	1.429	
CV	31.0	6.8	35.1	41.3	6.8	39.7	
HSL/aP2							
Mean	1.536	1.766	1.484	1.520	1.478	1.525	0.23
SD	0.184	0.356	0.091	0.143	0.078	0.199	(.0306)
Min.	1.257	1.426	1.353	1.347	1.384	1.257	
Max.	2.136	2.136	1.566	1.713	1.555	1.718	
CV	11.9	20.1	6.1	9.4	5.3	13.1	

Table 7. Descriptive statistics of the ratio of mRNA of the genes adjusted for β-actin by breed group.

Gene	All breeds	Texel	Rideau	Dorset	Texel-Dorset	Karakul	Prob. (MSE)
LPL/ACC							
Mean	3.054	1.365a	2.358ab	4.472b	3.460ab	2.940ab	0.04
SD	1.557	1.465	1.575	1.484	0.936	1.106	(1.746)
Min.	0.408	0.408	1.140	3.065	2.197	1.464	
Max.	6.794	3.052	5.081	6.794	4.814	4.148	
CV	51.0	107.3	66.8	33.2	27.1	37.6	
LPL/HSL							
Mean	1.038	0.327	1.001	1.160	1.356	1.062	0.15
SD	0.564	0.284	0.630	0.371	0.584	0.551	(.272)
Min.	0.146	0.146	0.376	0.545	0.788	0.389	
Max.	2.312	0.654	1.973	1.507	2.312	1.636	
CV	54.3	86.7	63.0	32.0	43.1	51.9	
LPL/aP2							
Mean	1.389	0.469a	1.286ab	1.706b	1.753b	1.362ab	0.025
SD	0.620	0.392	0.656	0.468	0.441	0.504	(.261)
Min.	0.191	0.191	0.623	0.895	1.374	0.696	
Max.	2.434	0.917	2.245	2.057	2.434	1.898	
CV	38.5	83.7	51.0	27.4	25.2	37.0	
ACC/HSL							
Mean	0.361	0.286	0.443	0.294	0.382	0.372	0.44
SD	0.139	0.072	0.181	0.135	0.061	0.173	(.019)
Min.	0.080	0.214	0.252	0.080	0.316	0.141	
Max.	0.705	0.358	0.705	0.431	0.480	0.613	
CV	38.5	25.0	40.8	46.1	16.1	46.5	
ACC/aP2							
Mean	0.499	0.413	0.605	0.427	0.514	0.502	0.57
SD	0.182	0.097	0.261	0.175	0.073	0.219	(.0348)
Min.	0.132	0.301	0.351	0.132	0.448	0.252	
Max.	1.023	0.470	1.023	0.566	0.626	0.787	
CV	36.5	23.5	43.1	41.0	14.1	43.6	
HSL/aP2							
Mean	1.419	1.451	1.373	1.504	1.369	1.413	0.89
SD	0.223	0.174	0.203	0.189	0.253	0.320	(.0574)
Min.	1.013	1.307	1.138	1.294	1.053	1.013	
Max.	1.789	1.645	1.658	1.706	1.744	1.789	
CV	15.7	12.0	14.8	12.6	18.4	22.6	

Table 8. Simple correlation coefficients between mRNA of ACC, LPL, HSL and AP2 genes. Unadjusted values above diagonal, and adjusted values for β-actin below diagonal.

Gene	ACC	LPL	HSL	aP2
ACC	-	0.38 (.07)	-.34 (.12)	-.36 (.09)
LPL	0.15 (.50)	-	-.27 (.21)	-.13 (.55)
HSL	0.01 (.95)	0.01 (.98)	-	0.50 (.02)
aP2	0.05 (.82)	0.31 (.14)	0.85 (.00)	-

Table 9. Simple correlation coefficients between mRNA of ACC, LPL, HSL and aP2genes (unadjusted) and growth and carcass measurements[1].

Measurement	ACC	LPL	HSL	aP2
Gain per day of age, g	-.23 (.28)	-.17 (.44)	0.19 (.38)	0.19 (.39)
Body cavity fat, %	-.13 (.56)	-.02 (.94)	0.01 (.95)	0.11 (.61)
Carcass tissue, %				
Subcutaneous fat (SCF)	-.05 (.81)	0.39 (.06)	-.02 (.92)	0.12 (.59)
Intermuscular fat (IMF)	0.10 (.64)	0.32 (.14)	-.08 (.70)	0.13 (.55)
Total carcass fat (TCF)	0.02 (.94)	0.42 (.04)	-.06 (.78)	0.15 (.51)
Muscle	-.13 (.55)	-.49 (.02)	0.21 (.33)	-.16 (.47)
Bone	0.31 (.15)	0.03 (.88)	-.30 (.16)	-.06 (.79)
Ration of carcass tissues				
Muscle:TCF	-.11 (.61)	-.51 (.01)	0.14 (.52)	-.21 (.34)
Muscle:SCF	-.12 (.58)	-.52 (.01)	0.10 (.64)	-.20 (.35)
Muscle:IMF	-.04 (.87)	-.42 (.04)	0.14 (.53)	-.24 (.27)
Backfat thickness, mm	-.05 (.81)	0.26 (.22)	0.26 (.22)	0.32 (.13)
Longissimus dorsi muscle				
Width	-.38 (.07)	-.44 (.03)	0.48 (.02)	0.26 (.22)
Depth	-.36 (.09)	-.11 (.63)	0.07 (.75)	0.16 (.47)
Area	-.32 (.14)	-.42 (.04)	0.26 (.23)	0.09 (.67)
Circumference	-.36 (.09)	-.51 (.01)	0.30 (.16)	0.15 (.50)

1-Probability of the coefficients being different from zero are shown in bracket.

Table 10. Simple correlation coefficients between mRNA of ACC, LPL, HSL and aP2 genes adjusted for β-actin and growth and carcass measurements[1].

Measurement	ACC	LPL	HSL	AP2
Gain per day of age, g	-.18 (.40)	-.14 (.53)	0.14 (.52)	0.09 (.67)
Body cavity fat, %	-.08 (.72)	-.14 (.51)	-.17 (.42)	-.21 (.34)
Carcass tissue, %				
Subcutaneous fat (SCF)	-.05 (.81)	0.33 (.12)	-.09 (.68)	-.15 (.48)
Intermuscular fat (IMF)	0.10 (.63)	0.28 (.18)	-.32 (.14)	-.32 (.14)
Total carcass fat (TCF)	0.02 (.92)	0.39 (.08)	-.23 (.30)	-.27 (.21)
Muscle	-.10 (.64)	-.41 (.05)	0.22 (.31)	0.25 (.25)
Bone	0.24 (.27)	-.06 (.77)	0.11 (.62)	0.10 (.65)
Ration of carcass tissues				
Muscle:TCF	-.08 (.73)	-.42 (.04)	0.20 (.35)	0.24 (.27)
Muscle:SCF	-.10 (.65)	-.42 (.04)	0.14 (.54)	0.19 (.38)
Muscle:IMF	0.00 (1.0)	-.38 (.07)	0.25 (.24)	0.25 (.25)
Backfat thickness, mm	0.07 (.74)	0.43 (.04)	-.19 (.38)	-.18 (.41)
Longissimus dorsi muscle				
Width	-.29 (.17)	-.23 (.27)	0.21 (.33)	0.14 (.52)
Depth	-.39 (.06)	-.08 (.71)	-.08 (.72)	-.05 (.82)
Area	-.31 (.14)	-.33 (.12)	0.04 (.84)	0.02 (.92)
Circumference	-.33 (.13)	-.37 (.08)	0.08 (.70)	0.02 (.91)

1-Probability of the coefficients being different from zero are shown in brackets.

iii. Relationships Between mRNA Levels of the Four Genes

Simple correlation coefficients between mRNA levels of the four genes, for both the unadjusted and ß-actin adjusted values, are shown in Table 8. Significant positive correlations were observed between HSL and aP2 levels for both unadjusted and ß-actin adjusted mRNA levels, with coefficients of 0.50 and 0.85, respectively. Moderate correlation coefficients existed between unadjusted ACC and LPL (0.38, P=0.07) and between unadjusted ACC and aP2 (-.36, P=0.09), which became smaller and lost significance when adjustments were made for ß-actin.

iv. Correlation Between Carcass Traits and mRNA Levels

Simple correlations between mRNA levels of the genes and carcass measurements were computed for both unadjusted (Table 9) and ß-actin adjusted (Table 10) values. With a few exceptions, relationships between carcass traits and levels of mRNA of all the genes were linear. Although ACC mRNA showed no relationship with carcass fat, it did show negative correlations with carcass muscle. The correlation coefficients between longissimus dorsi (LD) muscle width (-0.38), depth (-0.36), and circumference (-0.36) approached significance (P<0.10). The relationships became weaker when adjusted mRNA values were used, and only the correlation with LD muscle depth approached significance (-.39, P=0.06).

Both adjusted and unadjusted LPL mRNA values were positively correlated with the measures of carcass fat and negatively correlated with the measures of carcass muscle. The correlation coefficients between unadjusted LPL mRNA and percentage of carcass muscle and ratios of muscle to carcass fat ranged from -.42 to -.52 (P<0.05). Correlation

coefficients between LPL mRNA and the dimensions of LD muscle, except for LD muscle depth, were large (-.42 to -.51) and significant. Percentage of total carcass fat showed a stronger correlation with the LPL mRNA (0.42, $P<0.05$) than with each of its two components, i.e., subcutaneous fat (0.39, $P=0.06$) and intermuscular fat (0.32, $P=0.14$). Adjustment of LPL mRNA resulted in weaker relationships, but the general pattern did not change; the correlation coefficients between adjusted LPL mRNA and carcass muscle were positive and just significant (0.41, $P=0.05$), and those between the ratio of muscle to intermuscular fat (-.38,$P=0.07$), ratio of muscle to subcutaneous fat (-.42,$P<0.05$) and ratio of muscle to total carcass fat (-.42,$P<0.05$) were slightly smaller than the corresponding figures when unadjusted mRNA values were used. LD muscle dimensions showed negative but nonsignificant correlations with adjusted LPL mRNA; the largest coefficient was with LD muscle circumference (-.37, $P=0.08$). Adjustment of LPL mRNA resulted in an increase in the correlation coefficient with backfat thickness, from 0.26 to 0.43 ($P<0.05$).

The correlation coefficients of adjusted and unadjusted HSL mRNA and carcass muscle measurements were generally positive, and those with carcass fat measurements were all negative. The magnitudes of correlation coefficients were, however, all small and nonsignificant, except for the correlation between unadjusted HSL mRNA and LD muscle width (0.48, $P<0.05$). The OD for aP2 mRNA was not related to any of the carcass traits.

Tables 11 and 12 present simple correlation coefficients between the ratio of mRNA of the four genes and carcass traits. In most cases, the correlation coefficients

Table 11. Simple correlation coefficients between ratio of mRNA of the genes (unadjusted) and growth and carcass measurements[1].

Measurement	LPL/ACC	LPL/HSL	LPL/aP2	ACC/HSL	ACC/aP2	HSL/aP2
Gain per day of age, g	-.08 (72)	-.23 (.29)	-.23 (.29)	-.29 (.18)	-.31 (.15)	-.03 (.88)
Body cavity fat, %	0.05 (.83)	-.02 (.91)	-.04 (.85)	-.10 (.65)	-.14 (.54)	-.13 (.56)
Carcass tissue, %						
Subcutaneous fat (SCF)	0.39 (.06)	0.37 (.08)	0.35 (.10)	-.03 (.90)	-.07 (.75)	-.17 (.45)
Intermuscular fat (IMF)	0.20 (.34)	0.29 (.18)	0.26 (.23)	0.12 (.57)	0.05 (.84)	-.24 (.27)
Total carcass fat (TCF)	0.36 (.09)	0.39 (.06)	0.36 (.09)	0.05 (.82)	-.02 (.92)	-.23 (.28)
Muscle	-.36 (.09)	-.48 (.02)	-.42 (.04)	-.19 (.40)	-.07 (.74)	0.39 (.06)
Bone	-.11 (.63)	0.09 (.69)	0.05 (.82)	0.34 (.11)	0.29 (.18)	-.23 (.30)
Ration of carcass tissues						
Muscle:TCF	-.41 (.05)	-.50 (.01)	-.44 (.03)	-.15 (.50)	-.04 (.85)	0.38 (.07)
Muscle:SCF	-.43 (.04)	-.50 (.01)	-.45 (.03)	-.14 (.51)	-.06 (.79)	0.34 (.12)
Muscle:IMF	-.35 (.10)	-.40 (.06)	-.33 (.12)	-.08 (.71)	0.05 (.83)	0.41 (.05)
Backfat thickness, mm	0.22 (.32)	0.17 (.44)	0.15 (.49)	-.15 (.50)	-.19 (.39)	-.13 (.56)
Longissimus dorsi muscle						
Width	-.14 (.53)	-.54 (.01)	-.50 (.01)	-.50 (.01)	-.43 (.04)	0.17 (.45)
Depth	0.09 (.67)	-.14 (.52)	-.16 (.45)	-.33 (.12)	-.37 (.08)	-.10 (.64)
Area	-.21 (.33)	-.46 (.03)	-.43 (.04)	-.37 (.08)	-.33 (.12)	0.14 (.53)
Circumference	-.25 (.25)	-.56 (.01)	-.53 (.01)	-.43 (.04)	-.38 (.07)	0.12 (.59)

1-Probability of the coefficients being different from zero are shown in brackets.

Table 12. Simple correlation coefficients between ratio of mRNA of the genes (adjusted for β-actin) and growth and carcass measurements[1].

Measurement	LPL/ACC	LPL/HSL	LPL/aP2	ACC/HSL	ACC/aP2	HSL/aP2
Gain per day of age, g	-.07 (.76)	-.18 (.42)	-.15 (.48)	-.27 (.22)	-.23 (.28)	0.10 (.28)
Body cavity fat, %	0.17 (.44)	0.21 (.33)	0.21 (.33)	0.11 (.62)	0.08 (.70)	0.01 (.96)
Carcass tissue, %						
Subcutaneous fat (SCF)	0.33 (.13)	0.32 (.13)	0.40 (.06)	0.05 (.80)	0.07 (.76)	0.14 (.52)
Intermuscular fat (IMF)	0.21 (.34)	0.38 (.07)	0.46 (.03)	0.32 (.13)	0.31 (.15)	-.08 (.71)
Total carcass fat (TCF)	0.32 (.13)	0.41 (.05)	0.50 (.01)	0.20 (.35)	0.21 (.34)	0.05 (.82)
Muscle	-.31 (.14)	-.42 (.04)	-.52 (.01)	-.25 (.25)	-.25 (.24)	-.03 (.90)
Bone	-.14 (.51)	-.16 (.46)	-.13 (.54)	0.06 (.79)	0.11 (.63)	0.01 (.98)
Ration of carcass tissues						
Muscle:TCF	-.36 (.09)	-.46 (.03)	-.55 (.01)	-.23 (.29)	-.23 (.30)	-.03 (.86)
Muscle:SCF	-.36 (.09)	-.43 (.04)	-.52 (.01)	-.19 (.38)	-.21 (.34)	-.09 (.69)
Muscle:IMF	-.34 (.11)	-.44 (.04)	-.52 (.01)	-.21 (.34)	-.18 (.41)	0.06 (.82)
Backfat thickness, mm	0.23 (.13)	0.49 (.02)	0.54 (.01)	0.13 (.54)	0.12 (.58)	-.04 (.87)
Longissimus dorsi muscle						
Width	-.03 (.90)	-.28 (.19)	-.30 (.16)	-.40 (.06)	-.37 (.08)	0.12 (.59)
Depth	0.13 (.56)	0.02 (.93)	-.02 (.92)	-.21 (.33)	-.29 (.18)	-.11 (.62)
Area	-.16 (.48)	-.27 (.21)	-.33 (.12)	-.28 (.20)	-.30 (.17)	0.01 (.99)
Circumference	-.17 (.45)	-.34 (.12)	-.39 (.07)	-.32 (.13)	-.32 (.14)	0.07 (.74)

1-Probability of the coefficients being different from zero are shown in brackets.

between the ratios of unadjusted LPL over ACC, HSL and aP2 mRNA values and carcass traits were slightly smaller than the corresponding values when LPL mRNA was used. These values were positively related to the measures of carcass fat, and were significant or approached significance in the case of subcutaneous fat and total carcass fat. In contrast, these values were negatively correlated with the measures of carcass muscle, and were significant or approached significance in the case of percentage of carcass muscle, ratios of muscle to total carcass fat and subcutaneous fat, width, area and circumference of the LD muscle (Table 11). The correlation coefficients between dimensions of LD muscle and LPL/ACC were smaller, and those with LPL/HSL and LPL/aP2 were slightly larger than the corresponding coefficients when LPL mRNA was used.

The correlation coefficients between adjusted LPL/ACC mRNA values and carcass traits were smaller than the corresponding coefficients when adjusted LPL values were used, and were mostly nonsignificant. On the contrary, adjusted LPL/HSL and LPL/aP2 mRNA showed stronger correlations with carcass traits compared with adjusted LPL mRNA. This was particularly evident in the case of LPL/aP2, where the correlation coefficients with percentage of carcass muscle (-.52), muscle to carcass fat ratio (-.55), muscle to subcutaneous fat ratio (-.52), muscle to intermuscular fat ratio (-.52), backfat thickness (0.54), percentages of subcutaneous fat (0.40), intermuscular fat (0.46) and total carcass fat (0.50) were all considerably higher than the corresponding values when LPL mRNA was used.

The correlation coefficients between carcass traits and both adjusted and

Table.13. Regression equations of carcass measurements on unadjusted mRNA values[1,2].

Carcass Measurement	Independent variable	Regression coefficient	Standard error	Sig. level	Partial R^2, %[3]
Carcass fat measurements					
Subcutaneous fat (SCF), %	LPL*aP2	+13.1759	6.4883	0.055	16.4
Intermuscular fat (IMF), %	LPL*aP2	+8.9419	5.3910	0.112	11.6
Total carcass fat, %	LPL*ap2	+22.1178	9.7471	0.034	19.7
Backfat thickness, mm	LPL*aP2	+6.0554	3.4918	0.098	12.5
	HSL*aP2	+7.0012	4.2683	0.117	10.4
					(22.9)
Carcass muscle measurements					
Carcass muscle, %	LPL*aP2	-22.6241	8.3806	0.013	25.8
Longissimus muscle					
Width, cm	$(LPL)^2$	+4.0155	1.4647	0.011	29.1
	ACC*LPL	-8.2872	2.2568	0.002	9.3
	ACC*HSL	+2.4193	1.1366	0.047	11.9
					(50.3)
Depth, cm	$(ACC)^2$	-1.5784	0.7510	0.048	17.4
Area, cm^2	LPL	-25.5226	11.4834	0.039	21.7
	$(LPL)^2$	+32.7451	11.7997	0.012	8.2
	ACC*LPL	-15.7498	9.2980	0.107	14.5
					(44.4)
Circumference, cm	ACC*LPL	-5.5304	1.8096	0.006	30.8
Ratio of carcass tissues					
Muscle:total fat	LPL*aP2	-3.4362	1.1480	0.007	29.9
Muscle:SCF	LPL*aP2	-8.5671	2.8439	0.007	30.2
Muscle:IMF	LPL*aP2	-5.5483	2.2526	0.023	22.4

1-mRNA of the four genes, their squared values and cross-products (shown by asterisks) were used in stepwise regression procedures.
2-Gain per day of age, slaughter age, measures of body cavity fat, and % of carcass bone were not related to any of the measures of mRNA.
3-The model R^2 in multiple regression equations are shown in brackets.

Table 14. Regression equations of carcass measurements on mRNA values adjusted for β-actin[1,2].

Carcass Measurement	Independent variable	Regression coefficient	Standard error	Sig. level	Partial R^2, %[3]
Carcass fat measurements					
Subcutaneous fat (SCF), %	LPL	+1.8375	1.1283	0.118	11.2
Intermuscular fat (IMF), %	$(HSL)^2$	-0.7022	0.4230	0.112	11.6
Total carcass fat, %	LPL	+4.2090	1.6590	0.019	13.6
	aP2	-9.4006	4.3825	0.044	16.2
					(29.8)
Backfat thickness, mm					
	LPL	+3.9336	1.2332	0.005	18.5
	LPL*aP2	-1.7551	0.7380	0.028	18.0
					(36.5)
Carcass muscle measurements					
Carcass muscle, %	aP2	+8.8137	3.8435	0.033	16.9
	LPL*ACC	-6.2897	2.1548	0.009	17.3
					(34.2)
Longissimus muscle					
Width, cm	ACC	+2.8545	1.4024	0.056	12.8
	$(LPL)^2$	+0.5163	0.1728	0.008	16.4
	ACC*aP2	-3.2414	1.0048	0.004	12.7
					(41.9)
Depth, cm	$(ACC)^2$	-1.2489	0.5380	0.030	20.4
Area, cm^2	ACC	+12.7071	6.8477	0.079	18.3
	$(LPL)^2$	+2.2842	0.8439	0.014	13.2
	ACC*LPL	-14.9275	4.9060	0.007	10.5
					(42.0)
Circumference, cm	ACC	+6.9571	3.2827	0.047	21.4
	$(LPL)^2$	+1.2258	0.4045	0.007	14.1
	ACC*LPL	-8.0543	2.3518	0.003	12.3
					(47.9)
Ratio of carcass tissues					
Muscle:total fat	LPL	-0.7931	0.2243	0.002	17.9
	HSL	-1.1506	0.6320	0.085	15.4
	aP2	+2.9514	1.1097	0.016	9.9
					(43.2)
Muscle:SCF	LPL	-2.0111	0.5573	0.002	17.9
	$(HSL)^2$	-1.0320	0.4851	0.046	11.6
	aP2	+8.0422	2.8995	0.012	13.6
					(43.1)
Muscle:IMF	LPL	-1.0139	0.3894	0.017	14.8
	aP2	+2.1431	1.0286	0.050	15.2
					(30.0)

1-mRNA of the four genes, their squared values and cross-products (shown by asterisks) were used in stepwise regression procedures.

2-Gain per day of age, slaughter age, measures of body cavity fat, and % of carcass bone were not related to any of the measures of mRNA.

3-The model R^2 in multiple regression equations are shown in brackets.

unadjusted ACC/HSL and ACC/aP2 mRNA were all comparable, in both direction and magnitude, with the corresponding values between ACC mRNA and the same carcass traits. There were, however, notable increases in the magnitude of coefficients between unadjusted ACC/aP2 and ACC/HSL and LD muscle width (from -.38 to -.50 and -.43, respectively), between unadjusted ACC/HSL and LD muscle circumference (from -.36 to -.43), and between adjusted ACC/HSL and LD muscle width (from -.29 to -.40).

There was no relationship between adjusted HSL/aP2 mRNA and any of the carcass measurements, but unadjusted values were correlated with percentage of carcass muscle (0.39, P=.06), and muscle to intermuscular fat ratio (0.41, P=.05). Percentage of bone, gain per day of age and body cavity fat were not related to mRNA levels of any of the genes or their ratios.

The equations that best explained the variations in growth and carcass traits using unadjusted and adjusted mRNA levels are shown in Tables 13 and 14, respectively. Variations in gain per day of age, slaughter age, body cavity fat and percentage of bone were not related to any of the candidate gene mRNA levels at the 0.15 probability level, and therefore were not included in the Tables.

The product of unadjusted LPL and aP2 mRNA (LPL*aP2) best explained the variations in percentages of subcutaneous fat, intermuscular fat, total carcass fat, muscle, and ratios of muscle to total carcass fat, muscle to intermuscular fat and muscle to subcutaneous fat (Table 13). Each unit increase in LPL*aP2 resulted in 13.1759±6.4883 (P=0.055), 8.9419±5.3910 (P=0.11) and 22.1178±9.7471 (P=0.03) percent increase in subcutaneous fat, intermuscular fat and total carcass fat, explaining 16.4%, 11.6% and

19.7% of total variations, respectively. Each unit increase in LPL*aP2 resulted in 22.6241 ($P<0.01$) unit decrease in carcass muscle, and also in 3.4362 ($P<0.01$), 8.5671 ($P<0.01$) and 5.5483 ($P<0.05$) unit decrease in the ratios of muscle to total fat, subcutaneous fat and intermuscular fat, respectively. These equations explained 25.8%, 29.9%, 30.2% and 22.4% of total variations in percentage of muscle, ratios of muscle to total fat, Subcutaneous fat and intermuscular fat, respectively. The equation that best explained variations in backfat thickness included LPL*aP2 and HSL*aP2, both with positive coefficients, with R^2 value of 22.9%. The best regression models accounted for 50.3%, 17.4%, 44.4% and 30.8% of the variations in LD muscle width, depth, area and circumference, respectively. The common feature of the equations was that they all included a function of ACC, i.e., ACC*LPL, ACC*HSL or ACC^2, while aP2 did not appear in any of the models.

Adjusting mRNA for ß-actin did not result in equations with more explanatory power for percentages of subcutaneous and intermuscular fat, and for LD muscle width and area (Table 14). Only 11.2% and 11.6% of variations in subcutaneous and intermuscular fat were explained by adjusted mRNA of LPL and the square of HSL, respectively, with probability levels of close to 0.11. Approximately 30% of the variation in percentage of total carcass fat and the ratio of muscle to intermuscular were explained by a linear combination of adjusted LPL and aP2 mRNA levels. Each unit change in LPL and aP2 mRNA levels resulted in +4.2090 ($P<0.02$) and -9.4006 ($P<0.05$) percentage change in total carcass fat. The corresponding values for the ratio of muscle to intermuscular fat were -1.0139 ($P<0.05$) and +2.1431 ($P=0.05$). The equation for the

percentage of carcass muscle included aP2 (+8.8137, $P<0.05$) and LPL*ACC (-6.2897, $P<0.01$), explaining 34.2% of the variation.

Similarly, the adjusted mRNA values, equations for the dimensions of LD muscle all included functions of ACC. The variations in LD muscle area and circumference were best explained by equations which included ACC, LPL^2 and ACC*LPL, explaining 42% and 47.9% of variations. The equation for LD muscle depth included ACC, similar to that for the unadjusted mRNA.

v. Summary

Breed difference was significant only for unadjusted LPL mRNA, and the ratios of LPL/ACC and LPL/HSL. Differences among breeds was reduced when mRNA was adjusted for β-actin. Breeds were different only for LPL/ACC and LPL/aP2. In all the cases, the main contrast was between Texel and Dorset; the former had the lowest level of LPL mRNA, and the smallest ratios of LPL over the other gene's mRNA. LPL mRNA values, both adjusted and unadjusted, were positively correlated with the measures of carcass fat and negatively correlated with the measures of carcass muscle. ACC mRNA showed similar relationships with the LD muscle dimensions, and was not related to the other carcass traits. HSL and aP2 mRNA levels were not related to the carcass traits. In some cases, the ratio of the gene's mRNA improved the strength of the relationships with carcass traits over the mRNA of the gene with the strongest relationship. The variations in percentages of fat in carcass depots, carcass muscle, and their ratios were best explained by models that included the cross product of unadjusted LPL and aP2. Higher levels of LPL*aP2 resulted in higher fat and lower muscle percentages, explaining

Table 15 . Means of tail measurements and levels of mRNA of the genes by period.

Measurement	1	2	3	4	5	6	7	SEM[1]	Prob.
Body weight[2], kg	9.3 a	12.7 ab	15.9 bc	17.4 cd	20.2 d	26.7 e	33.8 f	1.40	0.000
Daily gain, g	165 ac	96 ab	113 ab	53 b	100 ab	232 c	178 ac	38	0.047
Tail thickness, cm	27.7 ab	27.8 ab	29.8 ab	25.2 a	32.0 b	38.2 c	48.4 d	2.12	0.000
Daily gain in tail thickness, mm	-	0.04 a	0.70 ab	-1.65 c	2.74 d	2.07 bd	2.56 d	0.55	0.000
Tail circumference, cm	22.9 ab	23.9 ab	24.0 ab	22.1 a	27.2 b	36.0 c	40.4 c	1.72	0.000
Daily gain in tail circumference, mm	-	0.28 abc	0.04 ab	-.68 a	1.82 cd	3.15 d	1.10 bc	0.56	0.008
Tail length, cm	19.8 a	22.7 b	23.3 bc	24.6 cd	25.4 d	27.5 e	29.8 f	0.48	0.000
Daily gain in tail length, mm	-	0.80	0.14	0.42	0.39	0.68	0.55	0.19	0.24
Unadjusted									
ACC	0.256 ab	0.396 bc	0.225 a	0.231 a	0.472 cd	0.543 d	0.463 cd	0.050	0.000
AP2	0.229	0.279	0.210	0.290	0.274	0.250	0.272	0.029	0.441
HSL	0.603	0.653	0.563	0.559	0.565	0.574	0.599	0.040	0.663
LPL	0.274 ab	0.441 bc	0.249 ab	0.145 a	0.659 c	0.624 c	0.252 ab	0.068	0.000
Adjusted									
ACC	0.425 a	0.592 ab	0.461 a	0.441 a	0.879 cd	0.978 c	0.735 bd	0.079	0.000
aP2	0.417	0.462	0.368	0.592	0.532	0.426	0.429	0.052	0.079
HSL	0.817	0.787	0.744	0.784	0.774	0.703	0.695	0.048	0.501
LPL	0.383 ab	0.626 bd	0.374 ab	0.206 a	0.895 c	0.781 cd	0.308 a	0.087	0.000

1- SEM: Standard error of the mean.
2- a,b,c,d denote significant differences among means within rows.

between 11.6% and 30.2% of variations.

B. Karkul Growth Trial

i.Growth Characteristics

Body weight, tail thickness, tail circumference and tail length all significantly increased as animals grew (Table 15). Lambs showed modest weight gains during period one (165 gd^{-1}, birth to April 19) and period 2 (96 gd^{-1}, April 20-May 24), when they had access to their mother's milk and creep feed. Lambs were turned onto a pasture on May 25, and showed the lowest gain (53 gd^{-1}) during period 4 (June 21-July 19), when pasture started to deteriorate while lambs' nutritional requirements were high. Rate of gain in period 5 was also low (100 gd^{-1}) due to weaning stress (lambs were weaned on July 19 and transferred to a feedlot), and change in the diet from pasture to dry feed. In addition, the lambs were fed a limited amount of grain during the first week in the feedlot, which was gradually increased to *ad libitum* within three weeks. The highest rate of gain (232 gd^{-1}) was realized in period 6, followed by that in period 7.

While gain in tail length did not change significantly over time, significant changes were seen over the seven periods for daily gain in tail thickness and tail circumference. Animals showed higher growth in tail thickness and circumference in the feedlot (periods 5, 6, 7) than in other periods. Tail thickness and circumference showed negative gain in period 4 (Table 15).

ii. Weight, Size and Tissue Composition of the Tail After Slaughter

Table 16 provides descriptive statistics for weight, percentage of tissues, length, and number of vertebrae in the tail of Karakul lambs studied. Average weight of tail,

Table 16. Descriptive statistics for weight, percentages of tissues, length and number of vertebrae in the Tail of Karakul lambs.

Measurement	Mean	Standard deviation	Minimum	Maximum	CV
Tail weight, g	1230	582	763	2237	47.3
Tail weight as % of cold carcass	7.45	1.87	6.19	10.76	25.1
Tail fat as % of tail weight	92.5	2.2	88.9	94.8	2.3
Tail muscle as % of tail weight	4.8	1.6	3.8	7.7	33.3
Tail bone as % of tail weight	2.6	0.8	1.4	3.3	30.7
Tail fat as % of carcass fat	23.8	3.0	21.3	27.9	12.6
Tail fat as % of subcutaneous fat	37.7	3.9	32.9	43.2	10.3
Length, cm	20.8	3.0	16.4	23.4	14.4
Number of vertebrae	9.5	1.7	7.0	11.0	17.9

Table 17. Simple correlations between tail measurements before (column headings) and after slaughter (Row headings).

	Slaughter Age	Body weight	Tail thickness	Tail circumference	Tail length
Tail weight	0.84 (0.08)	0.93 (0.02)	0.83 (0.08)	0.89 (0.04)	0.91 (0.03)
Muscle weight	0.39 (0.52)	0.65 (0.23)	0.53 (0.36)	0.83 (0.08)	0.63 (0.25)
Fat weight	0.83 (0.08)	0.94 (0.02)	0.84 (0.08)	0.89 (0.04)	0.91 (0.03)
Bone weight	0.55 (0.33)	0.46 (0.43)	0.18 (0.76)	0.16 (0.79)	0.65 (0.23)
Length	-.14 (0.82)	-.03 (0.96)	-.30 (0.63)	-.02 (0.96)	0.45 (0.44)
Number of vertebrae	-.27 (0.65)	-.17 (0.78)	-.43 (0.47)	-.15 (0.81)	0.32 (0.60)

Significance levels are shown in brackets

separated from the carcass after chilling, was 1230 g with values ranging from 582 g to 2237, and a coefficient of variation equal to 47.3%. Tail comprised 7.45% of the cold carcass weight, ranging from 6.19% to 10.76%, with a coefficient of variation of 25%. Fat comprised the main part of tail (92.5%), and only a small fraction of the tail was muscle (4.8%) or bone (2.6%). Tail weight was 23.8% and 37.7% of total carcass fat (subcutaneous plus intermuscular) and subcutaneous fat, respectively, with corresponding coefficients of variation of 12.6% and 10.3%. Average tail length was 20.8 cm (ranged from 16.4 to 23.4 cm), and the average number of vertebrae in the tail was 9.5 (ranged from 7 to 11). Weight of fat in the tail showed strong correlations with carcass subcutaneous fat ($r=0.988$, $P<0.01$), intermuscular fat ($r=0.947$, $P<0.05$), and total carcass fat (subcutaneous plus intermuscular, $r=0.981$, $P<0.01$).

iii. Relationships Between Tail Measurements Before and After Slaughter

Table 17 depicts simple correlation coefficients between age and weight at slaughter and tail dimensions before slaughter with weight, tissue composition and length of tail after slaughter. There was a strong correlation between slaughter weight and tail weight (0.93, $P<0.05$) and fat content of the tail ($r=0.94$, $P<0.05$). Heavier tails had larger circumference ($r=0.89$, $P<0.05$), thickness ($r=0.83$, $P=0.08$), and length ($r=0.91$, $P<0.05$) before slaughter. Older lambs had heavier slaughter weight ($r=0.95$, $P<0.05$), tail weight ($r=0.83$, $P=0.08$), tail fat ($r=0.83$, $P=0.08$), tail muscle ($r=0.39$, $P=0.52$), tail bone ($r=0.55$, $P=0.34$), but not a longer tail ($r=-.14$, $P=0.83$). The correlations between weight of fat in the tail and tail dimensions before slaughter were almost identical to

those between tail weight and tail dimensions before slaughter, an expected observation since 92.5% of tail weight was fat. Tail circumference was the only pre-slaughter tail dimension which was correlated with the weight of tail muscle ($r=0.83$, $P=0.08$). No significant correlations were observed between pre-slaughter dimensions of the tail and bone weight, bone length, or number of vertebrae.

iv. mRNA Levels During Growth

The mRNA levels of aP2 and HSL, both β-actin adjusted and unadjusted, did not vary over periods (Table 15). However, differences in mRNA levels during growth periods were seen in both unadjusted and β-actin adjusted data for ACC and LPL. The unadjusted and β-actin adjusted ACC mRNA showed a similar pattern over periods. The values were the lowest in periods 1, 3 and 4, intermediate in period 2, and the highest in periods 5, 6 and 7. Both unadjusted and adjusted ACC mRNA levels in periods 3 and 4 were significantly lower than those in periods 5, 6 and 7. The mRNA level in period 2 was significantly higher than those in periods 3 and 4 only in the case of unadjusted ACC mRNA. There was a slight decrease in ACC mRNA in period 7 compared with that in period 6, and the difference was significant only in the case of adjusted values.

Levels of unadjusted and adjusted LPL mRNA showed a similar pattern to ACC mRNA. There was a large but non-significant increase in mRNA levels from period 1 to period 2. Periods 3 and 4 showed the lowest levels of LPL mRNA, followed by a significant increased in periods 5 and 6. There was a sharp decline in LPL mRNA, both adjusted and unadjusted, from period 6 to period 7, resulting in the values in period 7 to be significantly lower than those in periods 5 and 6, and comparable to the values in

Table 18. Regression Coefficient of body weight, daily weight gain and tail measurements on unadjusted MRNA of each gene.[1,2]

Measurement	ACC	LPL	HSL	aP2
Body weight	-.134±5.820 (0.98)	-1.629 ±4.301 (0.71)	7.281 ± 7.055 (0.31)	10.908±9.747 (0.27)
Daily weight gain	0.154±0.154 (0.33)	0.152±0.112 (0.19)	0.239±0.189 (0.22)	0.504±0.250 (0.055)
Tail thickness	8.464±8.638 (0.34)	6.719±6.384 (0.30)	16.200±10.396 (0.13)	16.897±14.747 (0.26)
Daily gain in tail thickness	0.467±0.211 (0.04)	0.419±0.157 (0.02)	0.090±0.289 (0.76)	0.735±0.388 (0.074)
Tail length	-.271±2.00 (0.89)	0.608±1.480 (0.68)	1.598±2.462 (0.52)	6.233±3.192 (0.063)
Daily gain in tail length	0.030±0.082 (0.72)	0.081±0.062 (0.21)	0.045±0.101 (0.66)	0.078±0.147 (0.60)
Tail circumference	3.505±7.101 (0.63)	3.285±5.247 (0.54)	10.635±8.568 (0.23)	17.396±11.728 (0.15)
Daily gain in tail circumference	0.482±0.214 (0.04)	0.366±0.167 (0.04)	0.339±0.284 (0.25)	0.717±0.398 (0.087)

1-Significance levels are shown in brackets.
2- ± values represent standard error.

Table 19. Percentage of variation (partial R^2) in body weight, daily weight gain and tail measurements explained by unadjusted mRNA of each gene.

Measurement	ACC	LPL	HSL	aP2
Body weight	0.0	0.0	0.4	0.5
Daily weight gain	2.4	4.2	3.7	8.6
Tail thickness	0.7	0.7	1.5	0.9
Daily gain in tail thickness	5.8	7.6	0.1	4.5
Tail length	0.0	0.0	0.0	0.7
Daily gain in tail length	0.5	5.9	0.7	1.0
Tail circumference	0.1	0.2	0.8	1.2
Daily gain in tail circumference	8.1	7.7	2.7	5.6

Table 20. Regression Coefficient of body weight, daily weight gain and tail measurements on adjusted mRNA of each gene.[1,2]

Measurement	ACC	LPL	HSL	aP2
Body weight	-2.846±3.658 (0.44)	-3.033 ±3.305 (0.37)	-.441± 6.146 (0.94)	5.470±5.553 (0.33)
Daily weight gain	-.030±0.100 (0.76)	0.048±0.091 (0.60)	-.179±0.162 (0.28)	0.050±0.153 (0.75)
Tail thickness	3.794±5.558 (0.50)	2.305±5.074 (0.65)	3.169±9.987 (0.74)	2.667±8.569 (0.76)
Daily gain in tail thickness	0.250±0.142 (0.04)	0.267±0.128 (0.051)	-.022±0.271 (0.93)	0.292±0.242 (0.24)
Tail length	-1.505±1.237 (0.095)	-.039±1.159 (0.97)	0.117±2.117 (0.95)	3.232±1.832 (0.091)
Daily gain in tail length	-.014±0.053 (0.80)	0.051±0.048 (0.30)	0.037±0.094 (0.70)	0.024±0.088 (0.79)
Tail circumference	2.399±4.518 (0.60)	0.563±4.125 (0.89)	5.097±7.464 (0.50)	7.903±6.754 (0.25)
Daily gain in tail circumference	0.279±0.142 (0.065)	0.247±0.133 (0.078)	0.304±0.266 (0.27)	0.231±0.249 (0.37)

1-Significance levels are shown in brackets.
2 - ± values represent standard error .

periods 1, 3 and 4. This decline in LPL mRNA from period 6 to 7 was much larger than the decline observed for ACC mRNA.

v. Relationship of mRNA Levels of the Genes with Growth and Tail Measurements

Neither body weight nor the dimensions of tail showed any relationship with unadjusted (Table 18) or adjusted (Table 19) mRNA of the genes. The only exceptions were the regression of tail length on unadjusted and adjusted mRNA of aP2, which approached significance ($P<0.10$). These relationship are unlikely to be of any practical importance, as they explained a small proportion (0.7% and 0.6%) of the variations in tail length (Tables 20 and 21).

Daily gains in tail thickness and circumference were positively and significantly ($P<0.05$) related to unadjusted ACC and LPL mRNA levels, explaining between 5.8% to 8.1% of variation in these measurements. The same relationships were significant or approached significance in the case of adjusted mRNA values. The proportion of variation in tail thickness and circumference explained by the adjusted mRNA values were approximately 2% points smaller than those for unadjusted values (3.9% to 6.2%, respectively). Unadjusted aP2 mRNA also showed positive relationships ($P<0.10$) with gains in body weight, tail thickness, and tail circumference (Table 18), with partial R^2 values of 8.6%, 4.5%, and 5.6% respectively (Table 20). HSL mRNA was not related to any of the traits studied. Daily gain in tail length was the only measure of growth rate that was not related to mRNA levels of any of the genes.

Table 21. Percentage of variation (partial R^2) in body weight, daily weight gain and tail measurements explained by adjusted mRNA of each gene.

Measurement	ACC	LPL	HSL	aP2
Body weight	0.2	0.3	0.0	0.4
Daily weight gain	0.2	0.7	2.9	0.3
Tail thickness	0.3	0.2	0.1	0.1
Daily gain in tail thickness	3.9	5.2	0.0	2.0
Tail length	0.3	0.0	0.0	0.6
Daily gain in tail length	0.2	4.0	0.6	0.3
Tail circumference	0.2	0.0	0.3	0.8
Daily gain in tail circumference	6.2	5.9	0.5	1.6

Table 22 . Means of cross-product of mRNA of the genes by period.

Measurement	1	2	3	4	5	6	7	SEM	Pr.
Unadjusted									
ACC*LPL	0.074 ab	0.185 b	0.054 ab	0.034 a	0.347 c	0.346 c	0.124 ab	0.047	0.000
ACC*HSL	0.153 a	0.265 b	0.127 a	0.131 a	0.272 b	0.312 b	0.274 b	0.037	0.003
ACC*aP2	0.061 ab	0.116 ac	0.052 b	0.062 ab	0.133 d	0.137 d	0.128 cd	0.021	0.019
LPL*HSL	0.164 ac	0.294 ab	0.138 c	0.086 c	0.383 b	0.360 b	0.150 c	0.046	0.000
LPL*aP2	0.062 ac	0.128 ab	0.048 ac	0.043 c	0.190 b	0.157 b	0.069 ac	0.029	0.006
HSL*aP2	0.136	0.188	0.122	0.162	0.150	0.143	0.163	0.026	0.645
Adjusted									
ACC*LPL	0.176 ab	0.392 a	0.168 ab	0.075 b	0.811 c	0.769 c	0.246 ab	0.096	0.000
ACC*HSL	0.346 a	0.481 ab	0.343 a	0.350 a	0.677 bc	0.698 c	0.516 abc	0.068	0.002
ACC*aP2	0.189 a	0.297 ab	0.186 a	0.262 a	0.465 c	0.436 bc	0.327 abc	0.055	0.007
LPL*HSL	0.307 ab	0.510 ac	0.273 b	0.162 b	0.684 c	0.546 c	0.216 b	0.077	0.000
LPL*aP2	0.160 abc	0.312 acd	0.126 b	0.109 b	0.451 d	0.333 cd	0.133 ab	0.063	0.004
HSL*aP2	0.352	0.373	0.275	0.470	0.396	0.303	0.300	0.046	0.089

SEM: Standard error of the mean

Table 23. Regression of body weight, daily weight gain and tail measurements on cross-product of unadjusted mRNA of the genes.[1,2]

Measurement	ACC*LPL	ACC*HSL	ACC*aP2	LPL*HSL	LPL*aP2	HSL*aP2
Body weight	-1.199±6.187 (0.84)	4.770 ±7.881 (0.55)	7.041±13.583 (0.61)	0.229±6.351 (0.97)	1.558±10.077 (0.88)	15.67±10.95 (0.17)
Daily weight gain	0.179±0.163 (0.28)	0.272±0.207 (0.20)	0.626±0.346 (0.083)	0.237±0.165 (0.16)	0.432±0.258 (0.107)	0.592±0.284 (0.048)
Tail thickness	9.382±9.175 (0.32)	17.94±11.44 (0.13)	35.315±19.341 (0.081)	13.336±9.211 (0.16)	21.78±14.58 (0.149)	28.23±16.28 (0.096)
Daily gain in tail thickness	0.574±0.220 (0.017)	0.580±0 290 (0.060)	1.487±0.441 (0.003)	0.565±0.229 (0.024)	1.104±0.336 (0.004)	0.727±0.433 (0.11)
Tail length	0.636±2.129 (0.77)	1.106±2.726 (0.69)	4.348±4.616 (0.35)	1.453±2.166 (0.51)	3.292±3.403 (0.34)	6.650±3.667 (0.075)
Daily gain in tail length	0.101±0.087 (0.26)	0.063±0.111 (0.58)	0.140±0.192 (0.47)	0.120±0.088 (0.19)	0.143±0.144 (0.33)	0.090±0.161 (0.58)
Tail circumference	4.430±7.539 (0.56)	10.496±9.494 (0.28)	23.480±16.025 (0.15)	8.283±7.596 (0.29)	15.18±11.95 (0.21)	24.48±13.05 (0.073)
Daily gain in tail circumference	0.527±0.231 (0.034)	0.718±0.279 (0.019)	1.392±0.468 (0.008)	0.594±0.231 (0.018)	0.974±0.364 (0.015)	0.879±0.427 (0.053)

1- Significance levels are shown in brackets.

2- ± values represent standard error .

Table 24. Percentage of variation (partial R^2) in body weight, daily weight gain and tail measurements explained by cross-product of unadjusted mRNA of the genes.

Measurement	ACC*LPL	ACC*HSL	ACC*aP2	LPL*HSL	LPL*aP2	HSL*aP2
Body weight	0.0	0.1	0.1	0.0	0.0	0.7
Daily weight gain	2.8	4.0	7.1	4.7	6.2	9.1
Tail thickness	0.7	1.5	2.0	1.3	1.4	1.8
Daily gain in tail thickness	7.4	4.9	10.5	6.8	10.2	3.6
Tail length	0.0	0.0	0.2	0.1	0.2	0.6
Daily gain in tail length	4.7	1.2	1.9	6.4	3.5	1.1
Tail circumference	0.2	0.7	1.1	0.7	0.9	1.8
Daily gain in tail circumference	8.2	9.8	12.1	9.9	10.4	7.0

Table 25. Regression of body weight, daily weight gain and tail measurements on cross-product of adjusted mRNA of the genes.[1,2]

Measurement	ACC*LPL	ACC*HSL	ACC*aP2	LPL*HSL	LPL*aP2	HSL*aP2
Body weight	-3.207±2.954 (0.29)	-2.171 ±4.232 (0.61)	-1.601±5.271 (0.76)	-3.474±3.697 (0.36)	-2.511±4.605 (0.59)	6.442±6.147 (0.31)
Daily weight gain	-.009±0.082 (0.92)	-.093±0.114 (0.42)	-.040±0.143 (0.78)	-.009±0.102 (0.93)	0.062±0.125 (0.62)	-.034±0.170 (0.84)
Tail thickness	2.629±4.554 (0.57)	5.826±6.332 (0.37)	8.833±7.786 (0.27)	2.457±5.684 (0.67)	4.529±6.958 (0.52)	6.670±9.429 (0.48)
Daily gain in tail thickness	0.244±0.112 (0.043)	0.232±0.166 (0.18)	0.471±0.193 (0.025)	0.236±0.143 (0.12)	0.447±0.163 (0.013)	0.434±0.277 (0.13)
Tail length	-.848±1.028 (0.42)	-1.392±1.437 (0.34)	-.426±1.817 (0.82)	-.309±1.296 (0.81)	0.373±1.594 (0.81)	3.791±2.017 (0.073)
Daily gain in tail length	0.023±0.044 (0.60)	-.011±0.061 (0.86)	-.007±0.077 (0.92)	0.044±0.053 (0.42)	0.031±0.067 (0.65)	0.043±0.103 (0.68)
Tail circumference	0.995±3.708 (0.79)	5.185±5.107 (0.32)	8.771±6.214 (0.17)	1.515±4.610 (0.76)	3.748±5.631 (0.51)	12.996±7.226 (0.085)
Daily gain in tail circumference	0.252±0.114 (0.039)	0.378±0.154 (0.024)	0.427±0.202 (0.048)	0.302±0.140 (0.043)	0.378±0.176 (0.044)	0.514±0.275 (0.078)

1- Significance levels are shown in brackets.

2- ± values represent standard error .

vi. Ratio of mRNA of the Genes

The measurements taken on lambs were regressed on various ratios of ACC, aP2, HSL, and LPL mRNA. None of the equations was significant, and thus were not reported. The only exceptions were the regressions of daily gain in tail thickness on unadjusted ACC/LPL (0.2372 ±0.1269, P=0.077, R^2=4.4%), unadjusted LPL/HSL (0.2332 ±0.0974, P=0.027, R^2=6.5%), and adjusted LPL/HSL (0.1820 ±0.0924, P=0.064, R^2=4.8%).

vii. Relationships of Cross-Products with Growth Characteristics

Except for HSL*aP2, the cross-products of the adjusted and unadjusted mRNA of the genes were all significantly different across periods (Table 22). While the levels of significance varied among the cross-products of the gene's mRNA, all exhibited a similar pattern. Increases were seen from period 1 to period 2, low values in periods 3 and 4, a sharp increase in period 5 and 6, followed by slight (ACC*HSL, ACC*aP2), or sharp (LPL*HSL, LPL*ACC, LPL*aP2) drops in period 7. The values in periods 3 and 4 were significantly smaller than those in periods 5 and 6 in all the cases (except HSL*aP2). Body weight did not show any relationship with any of the cross-products of either adjusted or unadjusted mRNA of the genes (Tables 23 and 24). Adjusted and unadjusted HSL*aP2, which did not show significant variations among growth periods, were the only cross-products that were significantly (P<0.10) related to the actual tail measurements (thickness, circumference, length), with the exception of tail thickness on adjusted mRNA values. The partial R^2 values were all less than 2%.

Daily gain in tail circumference was positively and significantly (P<0.05) related to cross-products of unadjusted mRNA of all the genes, explaining between 7.0%

(HSL*aP2) to 12.1% (ACC*aP2) of the variations (Tables 23 and 25). The corresponding relationships with adjusted mRNA were significant or approached significance (HSL*aP2), but the R^2 values were somewhat smaller (Tables 24 and 26). Daily gain in tail thickness was also positively and significantly related to cross-products of unadjusted mRNA of all the genes, except for HSL*aP2. The cross-products explained 4.9% (ACC*HSL) to 10.5% (ACC*aP2) of the variations in daily gain in tail thickness. Adjusting mRNA reduced the number of significant regressions, and only those of daily gain in tail thickness on ACC*LPL, ACC*aP2 and LPL*aP2 remained significant at $P<0.05$ level, and explained 5.6%, 6.7% and 8.0% of the variations.

Significant regressions were observed for daily weight gain on unadjusted ACC*aP2 ($P=0.08$) and HSL*aP2 ($P<0.05$), (Table 23), with partial R^2 values of 7.1% and 9.1%, respectively (Table 24). The relationships became nonsignificant when cross-products of β-actin adjusted mRNA levels were used (Tables 25 and 26).

C. Comparison of Northern and Slot Blots

Figures 5, 6, and 7 show comparisons of OD values obtained from identical samples on northern and slot blots for β-actin, ACC, and LPL. In both ACC and LPL comparisons, the pattern of mRNA levels on northern blots are consistent with the pattern shown on slot blots. The differences seen in magnitude are likely the result of differences in exposure time.

The northern and slot blots for β-actin did not exhibit similar patterns. However, OD values for each sample appear comparable between blots.

Table 26. Percentage of variation (partial R^2) in body weight, daily weight gain and tail measurements explained by cross-product of unadjusted mRNA of the genes.

Measurement	ACC*LPL	ACC*HSL	ACC*aP2	LPL*HSL	LPL*aP2	HSL*aP2
Body weight	0.4	0.1	0.0	0.3	0.1	0.0
Daily weight gain	0.0	1.6	0.2	0.0	0.6	0.1
Tail thickness	0.2	0.6	0.8	0.1	0.3	0.3
Daily gain in tail thickness	5.6	2.6	6.7	3.5	8.0	3.2
Tail length	0.1	0.2	0.0	0.0	0.0	0.6
Daily gain in tail length	1.0	0.1	0.0	2.5	0.8	0.6
Tail circumference	0.0	0.6	1.1	0.1	0.3	1.6
Daily gain in tail circumference	7.8	9.2	7.3	7.6	7.5	5.9

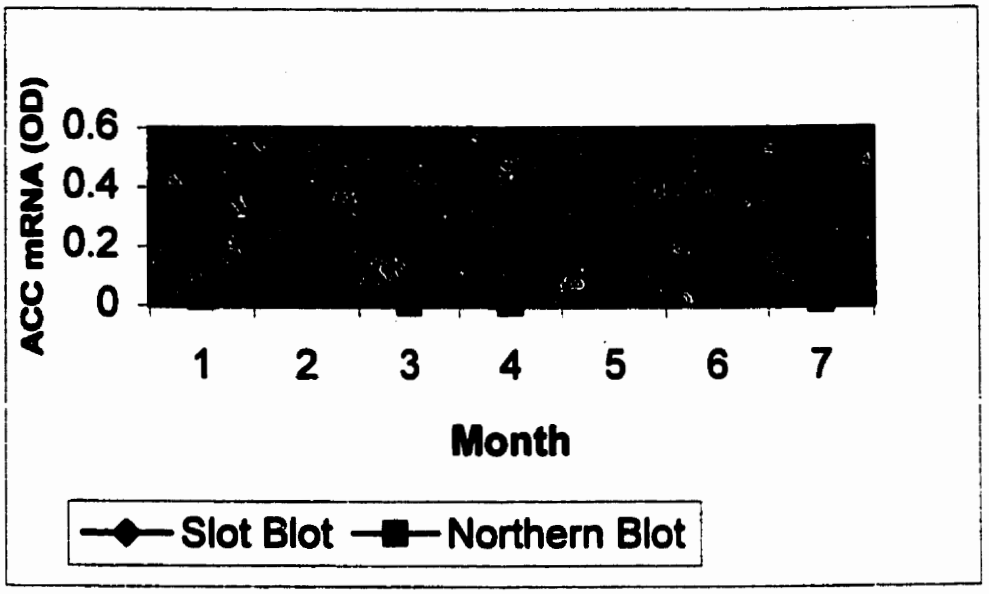

Figure 6. Comparison of OD values from northern and slot blots for ACC mRNA

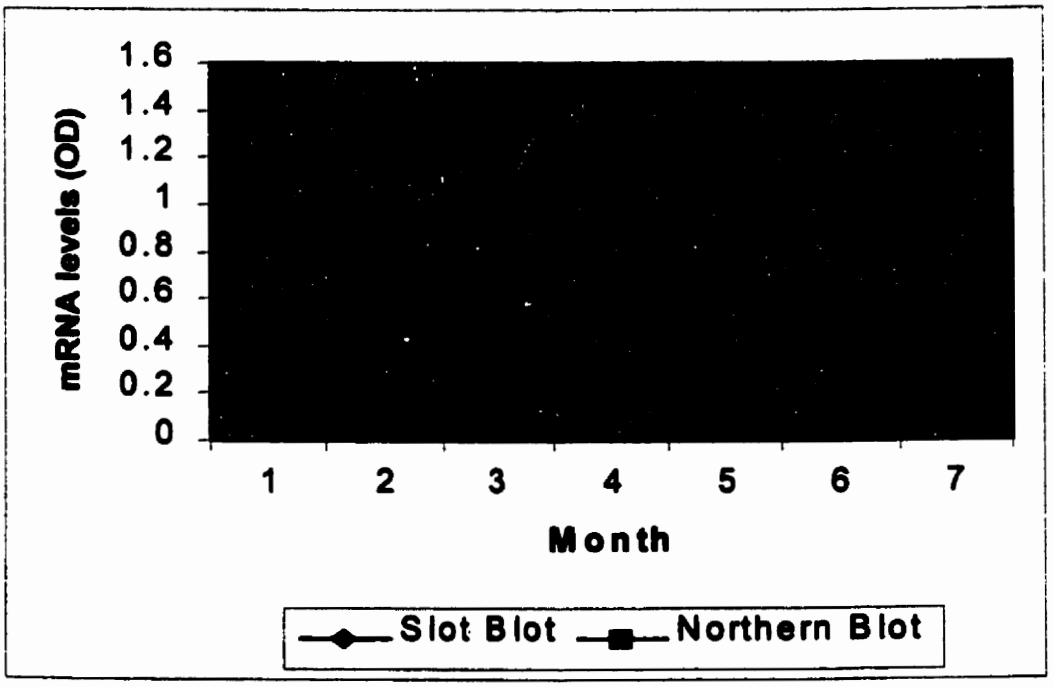

Figure 7. Comparison of OD values from northern and slot blots for LPL mRNA

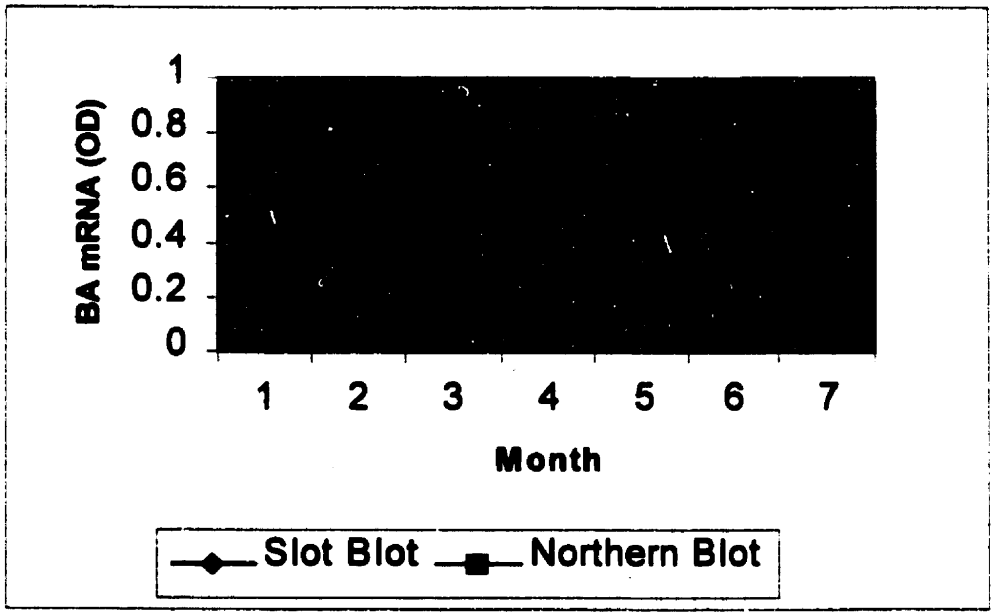

Figure 8. Comparison of OD values from northern and slot blots for β-actin mRNA

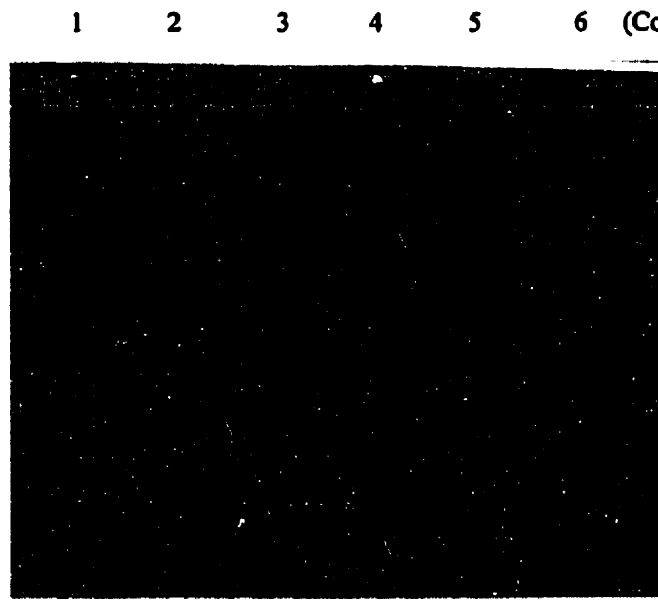

Figure 9.Autoradiograph of slot blot probed with radiolabeled LPL probe. Each column contains 8 serial dilutions of the same sample (tcRNA isolated from subcutaneous fat samples obtained for the Carcass Composition Trial; Column 1 to 6: Karakul, Texel, Internal Standard, Dorset, Texel-Dorset, Rideau)

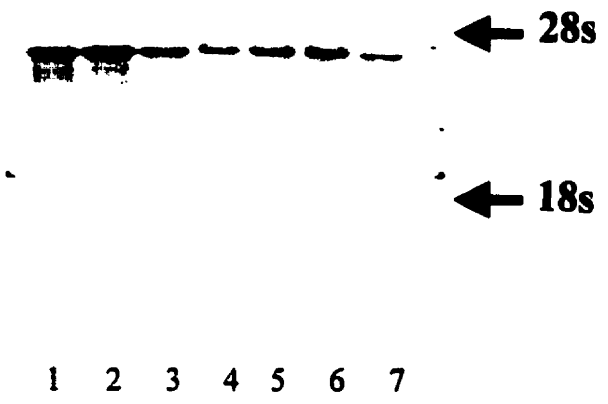

Figure 10. Autoradiograph of Northern Blot probed with a radiolabeled LPL probe
(tcRNA isolated from a lamb used in the Karakul Growth Trial; Left to Right: Periods 7-1)

5. DISCUSSION

A. Adjustments of mRNA Using ß-actin mRNA Levels

The same amount of total RNA of each sample was used in serial dilution, and the OD value at 1 μg of total RNA was calculated for each sample. Therefore, the OD values represent the amount of mRNA of each specific gene that existed in one μg of total RNA, and are thus useful values for comparative purposes. However, variations may occur in measurements due to inaccuracies in quantitation and/or uneven loading among samples. Therefore, conventional methods of reporting mRNA levels have always involved the use of a "house keeping " gene as an internal control. This transcript, usually a ribosomal RNA or a structural mRNA (such as the actins), is believed to be constitutively expressed in the tissue studied (Sambrook *et al.*1989). Adjusted values thus represent the relative amounts of mRNA of a gene per unit of ß-actin. For this reason, they provide a baseline measurement of the number of cells present in the RNA sample, and thus should reduce variations in the data that may be caused by inaccurate quantification and/or loss of sample during the quantification process.

Spanakis (1993) has suggested that these "housekeeping" genes can also be subjected to the same variability as mRNA of interest. For example, Vallett *et al.* (1993) found rRNA transcription in cultured *Drosophila* cells change rapidly in response to the addition of tumor-promoting phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (TPA) or serum. Haim-Muchnik *et al.* (1986) found transcription of liver rRNA varied significantly in response to differences in nutritional management. To ensure that this was not the case in the

current experiment, differences in β-actin levels across breeds (Carcass Composition Trial) and periods (Karakul Growth Trial) were investigated. No significant differences were found, suggesting β-actin levels were an acceptable internal control.

However, such adjustments may increase variation in the data. Adjustments are made by dividing the OD value of a gene's mRNA by the OD value of ß-actin, resulting in the quantity of mRNA of a gene per unit of ß-actin. Any inaccuracy in measuring ß-actin will further escalate the inaccuracy in the adjusted mRNA in a sample. Since no significant differences in β-actin mRNA existed across breeds (Carcass Composition Trial) and periods (Karakul Growth Trial), it would suggest that the differences seen in mRNA of the four candidate genes were due to actual variations in these mRNAs, and not variations due to uneven loading amounts. For this reason, the original mRNA data of the genes, as well as their ß-actin adjusted levels were used for analysis.

B. Comparison of Northern and Slot Blots

The slot blot technique provides the user with a quick and easy method for the quantitative assessment of mRNA levels. The technique also allows for the processing of a large number of samples in a short period of time. Unfortunately, improper use of this technique can result in inaccurate results. Sambrook *et al.* (1989) cites improper technique as the main drawback of the system. Northern blots involve the partial purification of the mRNA of interest by gel electrophoresis. The detection of a single band ensures that the researcher has performed the protocol properly. However, it can be difficult to obtain autoradiographs in which all samples present are neither over or under exposed.

In the current study, the processing of a large number of blots made the slot blot technique the most favorable method. Unfortunately, northern blots revealed that background existed in addition to the band of interest. This background may have been the result of long run times at low voltages (11 to 12 hours at 30v), which may have cause sample degradation within the northern blot, although this seems unlikely as ethidium bromide staining showed strong 18 s and 28 s ribosomal bands. It may have also been due to the use of random primed probes, with smaller pieces of probe increasing the likelihood of non-specific binding.

The comparison of the OD values obtained from the northern and slot blots were an attempt to ensure that results obtained by slot blot were comparable to results obtained from northern blots. In both ACC and LPL, the similar patterning in northern and slot blots suggest that the slot blots were a reasonable assessment of mRNA levels. While the pattern differed slightly in the β-actin blots, the small range of differences seen in OD values across blots suggest this too was a reasonable assessment of mRNA.

C. Carcass Composition Trial

i. Investigation of Candidate Genes

a. Lipoprotein Lipase

The results of this experiment suggest that LPL is correlated with percentages of fat and muscle in a carcass. Texel, the leanest breed studied, had lower LPL mRNA levels when compared to the Dorset, the fattest breed, and Texel-Dorset cross. The exception to the correlation was the Texel-Dorset cross that showed significantly higher levels than the purebred Texel, although percentages of carcass muscle and fat were midway between the

values for the two breeds. There appears to be high percentages of heterosis shown by the Texel-Dorset cross for the unadjusted and adjusted LPL mRNA (52.4% and 43.8%, respectively). Similar values for Texel-Dorset cross and purebred Dorset for unadjusted (0.596 and 0.595, Table 4) and adjusted (1.901 and 1.941, Table 5) LPL mRNA suggests that the gene shows complete dominance, i.e., the allele(s) for the high LPL mRNA in Dorset being dominant over the allele(s) causing low values in Texel.

Simple correlations (Table 9 and 10) between LPL mRNA and carcass traits provide more evidence supporting the hypothesis that LPL is an indicator of carcass composition. Positive, and mostly significant, correlation coefficients between unadjusted and adjusted LPL mRNA levels and measures of carcass fat, and negative correlations with percentage of carcass muscle and muscle to fat ratios point to the increased levels of LPL mRNA as a key indicator of increased carcass fat content or percentage.

The results support the concept of a pivotal role for LPL in the uptake of circulating fatty acids, and in the regulation of fat deposition. Elevated levels of LPL mRNA have been noted in several lines of obesity prone mice (Fried *et al.* 1991, Pagliassotti *et al.* 1994, Dugail *et al.* 1992, Eckel 1989). Some researchers speculated that the elevated LPL levels may be due to a mutation in another gene controlling/affecting LPL production (Fried *et al.* 1991, Eckel 1989). However, recent work suggests that LPL's role in fat metabolism may be direct. Jemma *et al.* (1995) found polymorphisms in the LPL gene which are linked to increased body mass index in humans. The authors suggested that this allele, which is also present in a small percentage of normal subjects, may provide a predisposition for obesity.

Since this gene appears to play such a pivotal role, it might also be possible that a mutation could exist predisposing animals to lean meat production.

In contrast, Brockman *et al.* (1996) found no significant differences in LPL mRNA levels between obese and lean lines of mice. However, most researchers suggest that complex traits such as fat content have as many as 20 genes which could contain alleles capable of causing phenotypic changes (Sellier 1994). Therefore, the lack of significant difference in one species or line may simply indicate that LPL is not the malfunctioning gene in that system, but could play an important role in fat deposition in other species or breeds. Furthermore, most of the published information on LPL is in mice and humans, which are fundamentally different from ruminants in fatty acid synthesis. A high percentage of fat synthesis in ruminants takes place in adipocyte, while liver is the main site of fat synthesis in nonruminants (Vernon 1980, Vernon 1992).

LPL has been identified in both the heart and skeletal muscles (Ladu *et al.* 1991) and plays a pivotal role in the delivery of energy to muscle tissue, regulating the supply of lipid for oxidative metabolism (Ranganathan *et al.* 1995). Muscle LPL is regulated in an inverse manner to adipose tissue LPL. For example, a 6-day fast caused mRNA levels in rat adipose tissue to decrease by 66%, but this same fast doubled levels in heart muscles and tripled levels in red vastus muscles (Ladu *et al.* 1991). Assuming that elevated levels of adipocyte LPL enzyme cause an increase in carcass fat content, the relationship of LPL with percentage of muscle is naturally negative, as we observed in the current study. Negative correlations between adipose LPL mRNA and LD muscle dimensions in this study, which were

significant or approached significance in several cases, indicate that LPL may have a direct effect on muscle content of lambs as well.

The relationships between LPL mRNA with LD muscle dimensions and the ratio of carcass tissues suggest that this gene is valuable for marker assisted selection. They also support the hypothesis of a key role for LPL in the development of both muscle tissue and carcass fat. The identification of alleles that cause the activity of the gene and enzyme to change could have a large impact in animal selection schemes.

b. Acetyl-CoA Carboxylase

ACC mRNA values were not different among breeds, nor did they show any significant relationship with percentages of carcass fat. In contrast, recent investigations into ACC mRNA levels in selected mouse lines have shown this gene product to be elevated in obese lines (Cheema and Clandinin 1996). Likewise, Sninnet-Smith and Woolliams (1988) found significantly lower ACC activity in the lean Texel when compared to Oxford, Soay, and East Friesian breeds. These reports suggested that ACC mRNA levels and enzyme activity is positively correlated with fat and negatively correlated with lean content. This is expected as ACC is the rate limiting step in fatty acid synthesis (Hardie 1989), catalyzing the first committed reaction in the *de novo* synthesis of fatty acids (Davey 1986).

ACC levels were negatively correlated with the LD muscle dimensions, and the regression coefficients were significant for depth (adjusted mRNA), and for width, depth, and circumference (unadjusted mRNA). LD muscle dimensions are positively correlated with the size of animal, as well as with total carcass muscle. Therefore, heavier carcasses

have larger LDs. Correlation coefficients between LD muscle width, depth, area and circumference with the side weight in our data were 0.78, 0.71, 0.81 and 0.83, respectively, all highly significant. These values suggest that LD muscle dimensions are more strongly related to carcass weight than with the carcass leanness (i.e., percentage of carcass muscle). However, the larger animals in the present experiment tended to have a higher percentage of muscle (r=0.34, p=0.11) and a lower percentage of fat (r=-.15, p=0.49), and were thus somewhat leaner than lighter animals. Therefore, larger LD dimensions reflected higher lean muscle percentages. The negative relationship between ACC mRNA and leanness is in agreement with the results of the experiment with obese mice (Cheema and Clandinin 1996). Work by Lu *et al.* (1994) showed injections of growth hormone reduced ACC mRNA levels in porcine adipose, again highlighting this negative relationship.

c.aP2

aP2 mRNA levels were not different among breeds nor was it significantly related to any of the carcass traits. Muggli-Cockett *et al.* (1992) found positive correlations (0.44, P<0.10) between aP2 mRNA (when adjusted for β-actin) and backfat thickness in lambs. The correlation coefficient between unadjusted aP2 mRNA and backfat thickness in the present work was 0.32 (P=0.13), which became smaller and negative when adjusted for ß-actin. The lack of information regarding the exact function of the aP2 gene within the adipocyte severely hampers any attempt to fully explain the differences seen between the two experiments.

d. Hormone Sensitive Lipase

HSL catalyzes the first two steps in TG breakdown, hydrolyzing the 1' and 3' position fatty acids (Vernon 1992, Yeaman 1990), and evidence suggests HSL is the key regulation point in lipolysis (Vernon 1992, Yeaman 1990, Fredrikson *et al.* 1981). As previously mentioned, this enzyme's activity is controlled by a complex array of intracellular signaling (Vernon 1992). The small variation in HSL mRNA amongst breeds suggests this gene is an unlikely candidate in the control of carcass fat content. Studies performed on both the pig (Liu *et al.* 1995) and the mouse (Sztalryd and Kraemer 1994) reported HSL mRNA levels were only affected after 2-3 days of fasting. Wilson *et al.* (1992) found significantly elevated levels of HSL mRNA during the hibernation of the yellow-bellied marmot. This information has led researchers to conclude that while transcriptional regulation of enzyme levels may occur after long term exposure to extreme conditions, control is most likely exerted by changes in enzyme activity and/or control exerted by other genes. The post-translation control of enzyme activity through phosphorylation and dephosphorylation provides the immediate mobilization of fatty acids supplying the necessary energy for the animal.

While lack of differences among breeds and the absence of any significant correlation with carcass tissues suggest that HSL would be a poor candidate gene for carcass composition, post-translational control could mean that mutations in the gene affecting enzyme activity could not be detected in the mRNA levels. However, a mutation in the translated area of the gene would seem unlikely, as it would cause changes in protein makeup and structure, and therefore may cause the enzyme to be dysfunctional

The only significant relationship between HSL mRNA and carcass traits was the positive correlation with LD muscle width, which would suggest increased HSL may supply increased energy for the muscles in heavy animals. While researchers have examined the relationship between HSL and fasting (Stzalyd and Kraemer 1994) and the consequences of an over-expression of HSL (Shimada *et al.* 1995, Stzaltyd *et al.* 1995), none related mRNA levels to muscle.

ii. Interactions of Candidate Genes

Since we are dealing with a complex metabolic system, the relative amounts of mRNA of the genes studied may play important roles in the control of carcass composition. Highly significant positive correlations were seen between both adjusted and unadjusted HSL and aP2 mRNA values. aP2 is a lipid trafficking protein, and responds to an increase in movement of hydrolyzed fatty acids within the adipocyte. The high and positive correlations of adjusted and unadjusted aP2 with HSL, and its negative correlations with adjusted ACC mRNA (Table 8), would suggest that aP2 is involved in fatty acid movement after lipolysis. However, this explanation appears to conflict with Muggli-Cockett *et al.* (1992), who found aP2 positively correlated with increased fat content. One difference between studies was that our animals were fasted overnight, and this may have caused an increase in HSL levels, which in turn caused an increase in free fatty acids, and higher aP2 levels. On the other hand, Sztalryd and Kraemer (1994) found HSL mRNA levels were only affected after 2-3 days of fasting.

The positive ACC and LPL correlation for unadjusted mRNA levels (Table 8) was

expected. Since ACC is the rate-limiting enzyme in *de novo* fatty acid synthesis and LPL regulates the uptake of circulating TG, it would be expected that these genes would both be high during periods of fat storage.

All possible ratios of mRNA of the genes were tested to investigate the relative amounts of mRNA of these genes among the breed groups. Not surprisingly, breed differences were significant or approached significance ($P<0.15$) for the ratios containing LPL. Texel, the leanest breed studied, had the smallest means for adjusted and unadjusted LPL/ACC, LPL/HSL and LPL/aP2, while Dorset and Texel-Dorset had the largest values. This may be due to the LPL mRNA values that were significant in unadjusted values and approached significance in adjusted values (Tables 4 and 5) coupled with steady-state levels of the other mRNA, producing an outcome identical to LPL alone. However, there was a substantial decrease in the P- values when the LPL /ACC ratio were compared to the LPL and ACC genes separately. This was apparent in both adjusted and unadjusted values (Tables 6 and 7), and showed that the combination of these two genes was more informative than each one separately. Despite the fact that breed differences for LPL alone were less pronounced than LPL expressed as a ratio of ACC, the magnitude of the correlation coefficients of carcass traits were similar for LPL/ACC and when LPL was used alone. It is possible that the Texel has a significantly lower uptake of circulating TG to *de novo* fatty acid synthesis ratio than the other breeds, and a heavy dependence on *de novo* fatty acid synthesis for stored fatty acids. It has been suggested that LPL may supply 30-50% of the fatty acids used for adipose tissue storage (Vernon 1992), and thus a drop in LPL enzyme

would represent a large reduction in fatty acid uptake. The observation that the ratios of LPL mRNA over that of all other genes had the smallest values in Texel, and large values in Texel-Dorset and purebred Dorset, suggests that the Texel has not modified its levels of other genes to compensate for lower LPL. The high levels of LPL mRNA seen in the Texel-Dorset cross indicates that if a different allele of this gene is present in the Texel, it may not be dominant.

The significance of the LPL/aP2 ratio is difficult to interpret. aP2 is a lipid trafficking protein, and lower levels of LPL coupled with stable aP2 levels may indicate greater lipid movement out of the adipocyte.

Another mRNA ratio with stronger correlation coefficients with carcass traits than the individual genes was the unadjusted ACC/HSL, which showed strong negative correlations with LD muscle width, area and circumference. Adjusted ACC/HSL also showed a stronger negative correlation with LD muscle depth than either ACC or HSL mRNA. Considering that higher ACC levels would favour lipogenic activity, and lower HSL levels would reduce lipolytic activity, the net result may be a shift towards storing energy in the form of fat and away from lean muscle development. HSL/aP2 was positively related to muscle in this study. This means that when HSL relative to aP2 is high, less fat is deposited. This seems to be in direct conflict with the positive correlation between HSL and aP2 mRNA levels. However, it may be that this ratio is high when HSL is higher than aP2 (HSL may be low, but aP2 could be lower, providing a high ratio).The aP2 gene could be reaching an upper limit, and might not increase to the same extent as the HSL. In these cases,

the larger increase in HSL simply reflects the animal's ability to mobilize fat quickly.

In conclusion, while differences among the breeds for the ratios of LPL mRNA levels over other gene mRNA levels were more pronounced than levels of transcripts of each of those genes alone, particularly for adjusted mRNA, the ratios did not generally show strong correlations with carcass traits. This may indicate that the ratios of different transcripts are more drastically affected over the breed development process than those of individual genes. Sinnett-Smith and Woolliams (1988) found the analysis of a combination of enzymes pointed to variations in fat metabolism among breeds. These authors found clear differences in fat metabolism across the four breeds they studied. Measurements of the incorporation of radiolabelled carbon into fatty acids, release of glycerol from adipose tissue, and ACC and LPL enzyme activity assays revealed that the sire breeds (Texel and Oxford) had a reduced capacity for *de novo* synthesis of fatty acids, when compared to the Soay (a feral breed) and the East Friesen (a dairy breed). The authors concluded that fatness did not appear to be related quantitatively or qualitatively to individual rates of *de novo* fatty acid synthesis, uptake of exogenous fatty acids, or hydrolysis. They found that a combination of the variation seen in all three aspects best described the fatness of the sheep. Due to the differences in fat metabolism among breeds, it may be concluded that the chance of finding mutations in a single gene which are associated with carcass composition is not high since several genes are likely affected. On the other hand, a clear marker of altered fat metabolism is conceivable.

iii. Regression Models Investigating Candidate Gene Relationships with Carcass Composition

Together, unadjusted LPL and aP2 mRNA accounted for 11.6% to 30.2% of the variation in the percentages of carcass fat (subcutaneous, intermuscular and total carcass fat), carcass muscle, and muscle to fat ratios. Similarly, the LPL and aP2 gene expression explained 11.2%-43.2% of the variation for these traits when β-actin adjusted values were used. The relationships between percentages and ratios of carcass tissues and LPL*aP2 were stronger than those for LPL. These findings show that the interaction between these two genes provided a more complete overview of activity within the fat depot. There was no correlation between unadjusted LPL and aP2 mRNA values ($r=-.13$, $P=0.44$, Table 8). However, positive regression coefficients reveal that fatter animals had higher LPL*aP2 values within the adipocyte compared with leaner animals. The strong relationship is consistent with the function of the two enzymes. LPL plays an important role in the uptake of circulating triglyceride (Eckel 1989), and aP2 has been shown to be involved in the trafficking of fatty acids within the cell (Matarese *et al.* 1989). The combined activity of these two genes could be providing a more complete view of fatty acid storage within the cell than either alone. Animals that have higher levels of both LPL and aP2 may be able to take up more fatty acids (higher LPL gene expression) and transport these fatty acids to fat droplets with the adipocyte (higher aP2 activity), resulting in animals which are capable of storing more fat.

Adjustment of mRNA values by dividing by β-actin mRNA expression changed the relationship between aP2 gene expression and carcass fat and muscle, but did not affect the relationships of other genes. It is unclear as to why the β-actin adjustment changes the aP2 relationships. It could indicate an interaction between β-actin and aP2, as the signs of correlation coefficients and regression coefficients of the other genes do not appear to be affected by β-actin adjustment.

Muggli-Cockett *et al.* (1992) found that an analysis of variance using breed and sex as fixed effects and LPL and aP2 mRNA as covariates showed both LPL and aP2 added significantly to a model for carcass yield grade (a United States Department of Agriculture grading system incorporating fat content). Contrary to the current study, simple correlations showed a negative link between LPL and backfat thickness, while aP2 was positively correlated.

Variations in the LD muscle dimensions were explained by rather complex equations. For β-actin adjusted values (Table 14), ACC, ACC^2, LPL, LPL^2 mRNA levels and the cross product of LPL*ACC mRNA levels explained large proportions of the LD dimensions, accounting for between 17.4% (depth) and 44.4% (width). Similarly, unadjusted mRNA (Table 13) for ACC, ACC^2, LPL, LPL^2 mRNA levels and the cross product of LPL*ACC mRNA levels explained large proportions of the LD dimensions, accounting for between 20.4% (depth) and 47.9% (circumference). The lower R^2 values shown by LD muscle depth, which also had the smallest correlations with total carcass muscle, could be caused by inaccuracies in measuring the LD muscle depth.

The ACC*LPL cross product had negative coefficients for loin width, area, and circumference, and suggests that animals with lower LD dimensions had higher ACC*LPL values. Unadjusted ACC and LPL mRNA levels were positively correlated (r=0.38, P=0.07, Table 8), and animals with higher levels of ACC and LPL produced a fatter carcass. Simple correlations between each of the LPL and ACC measurements with the LD muscle dimensions were negative. Increased mRNA expression of LPL and ACC in adipocyte tissues may require a diversion of energy from the muscle, which would explain the negative relationships observed in this study. The inclusion of LPL^2 in many of the LD regression equations improved R^2, and could indicate an upper limit to the amount of variation explained by LPL gene activity. Interestingly, neither the aP2 expression or the LPL*aP2 mRNA cross product, while important in determining percentages of the tissues and tissue ratios in carcasses, were significant in models describing LD dimensions. Overall, the results support previous studies that suggest ACC and LPL regulatory roles in fat metabolism, such that elevated expression of these genes in fat tissue may divert fuel away from the muscles to fat storage. However, aP2 mRNA levels appear to provide information regarding the activity of the fat depot only, suggesting they respond to signals received within the adipocyte.

ACC mRNA levels did not play an important role in any regression relating to fat content, but were related to muscle measurements. While the exact significance is unknown, ACC gene activity may provide information regarding energy partitioning, affecting lean content. Other factors within the adipocyte, such as LPL activity, may be masking its

importance in adipose tissue.

Gain per day of age, body cavity fat and percentage of bone were not related to the mRNA levels of any of the genes. Small variation in the sample for percentage of bone (CV=10.2), which was also shown in the lack of differences among breeds, could explain the absence of any relationship between mRNA of the genes and percentage of bone. Lack of any relationships between body cavity fat and gain per day of age with mRNA of the genes, despite the fact that significant breed differences existed for these traits, could indicate that the genes studied do not control rate of gain or fat metabolism in the non-carcass fat depots. These results could be considered as evidence supporting the validity of the results on carcass traits as well.

D. Karakul Growth Trial

i. Growth Characteristics

During the present experiment, lamb growth appeared to reflect the nutritional status of the animals. The highest gains were realized in periods when the animals were either on an *ad libitum* grain diet in the feedlot (periods 6 and 7) or had access to creep feed while nursing (period 1). Periods of low gains were observed when the animals' diet was restricted to pasture and the ewe's milk (periods 3 and 4), or after weaning and transition from pasture to dry feed (period 5). The non-significant decline in rate of gain during the last period in the feedlot (period 7), compared with that in period 6, could be partly due to the fact that the last weight was taken after 18 h of feed withdrawal, and partly because lambs' growth had reached a plateau.

The Karakul are a medium-size, multi-purpose (meat, milk, pelt, caper-wool), unimproved breed, originating from Western Asia, and were imported to North America in early 1900 (MacEwan 1941, Briggs and Briggs 1969). There is no indication that the breed has been selected for any economically important trait in this continent. The values for rate of gain observed in this experiment were low compared with those in improved breeds. Average daily gain from birth to weaning (90 days) in Karakul, Rambouillet, Suffolk x Rambouillet cross and Karakul x Rambouillet cross lambs were 283, 317, 304 and 282 gd^{-1}, respectively (Shelton *et al.* 1991). Pre-weaning rate of gain of Karakul lambs in that experiment was much higher than that the values observed in the present work. The authors attributed the high growth rate of Karakul lambs to a better milking ability of this breed. Post-weaning gains of Karakul, Rambouillet, Suffolk x Rambouillet cross and Karakul x Rambouillet cross in the same experiment (Shelton at al. 1991) were 159, 177, 170 and 168 gd^{-1}, respectively, consistent with the results of the present experiment.

Changes in tail circumference and thickness mirrored the lambs' growth performance, with periods of high daily weight gains coinciding with higher gains in both tail circumference and thickness. In period 4 (June 21-July 19) when pasture quality and quantity were low and lambs showed the lowest rate of growth, the tail circumference and thickness actually decreased. During this period, it is likely that these animals were in a negative energy balance, and the fat store was used to supply needed energy for the growth of vital organs, muscle and skeleton. Although the positive relationship between plane of nutrition and fat deposition is well documented (reviewed by Berg and Walters 1983), there are no

published reports on the changes in tail dimensions of fat-tailed sheep and plane of nutrition.

Tail is one of the major sites of fat reserve in fat-tailed breeds, and it seems logical to hypothesize that it is a very mobile fat depot, i.e. the first depot to which fat is deposited when surplus energy is available, and the first site from which fat is catalysed when the body requires energy. This hypothesis is based on the observation that kidney fat, which is the most mobile fat depot, is larger in dairy and adapted breeds of sheep and cattle, compared with that in meat-type breeds (Kempster *et al.* 1982; Berg and Walters 1983). The former breeds require a source of readily available energy during the peak of lactation, or during insufficient energy intake (Kempster *et al.* 1982). In their place of development in Western Asia, fat-tailed breeds can rapidly accumulate fat during the early spring when plenty of grass is available, and use up this reserve source of energy during the long and dry summer months when limited amount of feed is available. Furthermore, a large proportion of the fat-tailed breeds are managed under the transhumance systems, and travel up to several hundred kilometres from their summer pastures to winter ranges. Sheep under this system require a large energy reserve to survive periods of limited feed intake and long distance travel.

The highest daily gain in tail thickness was recorded in period 5, whereas the highest gain in tail circumference was recorded in period 6, and the second largest in period 5. Although statistically nonsignificant, this minor discrepancy could be due to the pattern of tail growth. Tail circumference is the function of depth and width, and it is possible that these two measurements did not grow proportionally. Kennedy *et al.* (1995) observed differences in growth of tail volume and tail circumference in clonidine-treated Karakul lambs, and

suggested that clonidine may affect the location of adipose tissue deposition within the tail. Inaccuracies in taking measurements on the tail may also be responsible for the observed differences. Attempts were made to take each measurement at the same location on the tail, and as consistently as possible.

Tail length grew consistently over this period of seven month, showing a different growth pattern compared with that in tail circumference and thickness. The reason for the observed differences between tail length and tail circumference and thickness is that tail length is primarily determined by the growth of skeleton, i.e., tail length increases at the same rate as caudal vertebrae. Short-term fat accumulation in the tail is manifested as increases in tail thickness and width (and thus circumference). Longitudinal growth of the tail is largely the result of increase in chronological age, because only a limited amount of fat is deposited at the tip of the caudal vertebrae, and short-term increase in tail length due to excessive energy is expected to be small. This is clearly seen in the daily gains in tail length in different periods (22.7 to 29.8 mm per day from 1 to 7 months of age).

It could be concluded from the results of this study that daily gains in tail circumference and thickness are accurate measures of the temporal nutritional status of the lamb, and rate of gain in tail length, on the contrary, is more indicative of chronological age and skeletal growth, and is less valuable as a measure of temporal nutritional status. The results also indicated that thickness and circumference of the tail more accurately reflect the energy intake profile than the body weight. This seems logical, although it has not been reported before, because animals primarily channel the available energy to the growth of

vital internal organs, skeleton and muscle. When an animal is under negative energy balance, it uses up the fat reserve to supply energy for the vital organs and tissues. Consequently, while the fat reserve is depleting, the animal shows some body weight gain.

There was a large degree of variability among the lambs for tail weight, ranging from 582 to 1230 g (Table 16), and this large variation was reflected in the large coefficient of variation (47.3%). Almost half of the variation was removed when tail fat was expressed as a percentage of cold carcass (CV=25.1%), which indicates that differences in the size of the animal contributed to the high degree of variability in tail weight. This is also shown by the high correlation coefficient between slaughter weight and tail weight (r=0.93, Table 17). The CV of 25.1% for tail weight as a percentage of carcass weight indicates that there is still considerable variation among animals for the amount of fat stored in the tail, even with carcass weight standardization. Part of this variability could be due to differences in lamb's age, sex, dam's milk production, and other environmental factors not accounted for by differences in slaughter weight. Differences among the lambs for genetic control of fat synthesis, and their tendency to store fat in the tail could have also contributed to the observed variation.

There is limited published information on the weight of tail in the Karakul breed. Average tail weight of Karakul lambs in Texas, slaughtered at an average weight of 47 kg was 1151 g or 4.5% of hot carcass weight (Shelton *et al.* 1991). Farid *et al.* (1983) reported the tail of culled old Karakul ewes to be 8.8% of chilled carcass, very close to 7.45% in the present study.

Karakul is one of many breeds of fat-tailed sheep, and large differences exist among breeds for the weight of fat-tail. For example, weight of fat-tail as a percentage of carcass weight is reported to be 3.7% to 4.6% in four groups of Awassi male lambs differing in initial feedlot weight and slaughtered at 50 kg (Al-Mahmood *et al.* 1976), 11.1% to 13.1% in Awassi lambs slaughtered at five different weight groups (35-55 kg) (Rashid *et al.*, 1987), 12.9% in Egyptian Ossimi lambs slaughtered at 50 weeks of age (Marai *et al.* 1987), 8.4% in South African Namaqua Afrikaner and its crosses with Pedi and Blackhead Persian (Joubert and Ueckermann 1971), and 13.2% to 16.4% in Kellakui lambs in four sex-nutritional treatment groups (O'Donovan *et al.* 1973). Tail weight as percentage of slaughter weight is reported to be 5.3% in Awassi culled ewes weighing 68 kg at slaughter (Epstein, 1982), 5.7% and 6.7% in 1.5 years old and 3 years old Egyptian Rahmani rams weighing 60 and 75 kg (El-Serafy *et al.* 1976), and 5.3% to 9.6% in feedlot finished lambs of three fat-tailed breeds (Awassi, Hamadani and Arabi) (Al-Jalili and Al-Wahab, 1985). These figures indicate that weight of tail in Karakul lambs in the present experiment was within the lower end of the tail weight in other breeds.

The observation that tail constituted 23.8% of total carcass fat and 37.7% of subcutaneous fat points to the importance of this depot as an energy store in fat-tailed sheep. An interesting observation was small differences among lambs for the proportion of total carcass fat and subcutaneous fat that was deposited in the tail. The small variations amongst lambs for tail fat as a percentage of total carcass fat (21.3% to 27.9%, Table 17) , and tail fat as a percentage of subcutaneous fat (32.9% to 43.2%), along with small coefficients of

variation (12.6 and 10.3, respectively) point to the prominent role that the tail plays in storing fat in the Karakul breed. The results clearly showed that tail operates as an extra reservoir for body fat, and is thus an accurate measure of total carcass fat and subcutaneous fat in this breed, as shown by the strong correlation coefficients between weight of fat in the tail and carcass fat measurements (r=0.95 to 0.988). There is no information in the literature on the percentages of tissues in the tail, or tail-fat as a percentage of fat in other depots to compare these results with.

Sheep have between 16 to 18 caudal vertebrae (Kempster *et al.* 1982). It was not possible to count the number of vertebrae without removing all the fat, making it difficult to separate the tail from the carcass exactly at the same spot in all the lambs. Therefore, the tail was severed at the intersection with the rest of the carcass. Length of the tail after slaughter did not show strong correlations with weight of tail (r=0.28, P=0.64), or weight of fat in the tail (r=0.27, P=0.66). While this may be due to the inaccuracies in separating the tail from the carcass, it may also simply point out that heavier tails were not necessarily the longest.

ii. Relationships Between Tail Measurements Before and After Slaughter

Strong and positive relationships between age and weight at slaughter and weight of tail and its fat content after slaughter indicate that older and faster-growing Karakul lambs accumulated more fat in their tail. The fact that rate of fat deposition increases as animals become older and heavier is well documented (Kempster *et al.* 1982; Berg and Walters 1983). The age and weight at which fat accumulation starts to accelerate depend on the breed

and life history of the animal. Although it is possible to alter carcass fat content by selection without a corresponding change in body weight (Cameron 1992), rapid fat accumulation at heavier weights in unimproved breeds is biologically understandable.

Among tail measurements before slaughter, tail length had the highest correlations with weight and fat content of the tail after slaughter. This would suggest tail length on live animals is an accurate predicator of weight and fat content of the tail. The strong correlations observed can probably be explained by the fact that the larger, more mature animals had longer tails, and thus would tend to have more fat stored, as they entered the fattening phase before the less mature, smaller animals. As previously discussed, it is the age and skeletal growth of the animal that are the primary affectors of tail length, as little fat is deposited at the tip of the tail. Therefore, tail length, which is an accurate measure of size of tail, is not a good measure of temporal changes in fat content of the tail. The length of the tail in the mature animal is expected to be static, while its fat deposits will be changing constantly as energy supply changes. There is no published information to compare these results with.

Both tail thickness and tail circumference measurements on the live animal showed strong correlations with both weight and fat content of the tail after slaughter. The correlation coefficients were only slightly smaller than that of tail length. Since tail thickness and circumference fluctuated throughout growth, and appeared to mirror the growth pattern of the animal (Table 15), the previous conclusion that tail circumference and thickness are the best live animal measurements to estimate tail fat content is reiterated.

It should be noted that attempts were made to measure the volume of the tail by water

displacement, as was performed by Gilson *et al.* (1995). This measurement proved to be too difficult and was abandoned. Measurements were not repeatable, as animals tended to thrash their tails displacing excessive water.

iii. mRNA Levels During Growth

The pattern of change of ACC mRNA levels over the growth periods showed a close relationship with the feeding regime of the growing lambs (Table 15). The lowest values were observed during the early age when lambs had not started to consume grain (period 1), and when lambs were placed on pasture and had access only to grass and the mother's milk (periods 3 and 4). High mRNA levels seen in periods 5 and 6 were obtained while animals were in a feedlot with *ad libitum* access to grain. The correlation between ACC mRNA and carbohydrate intake has been reported numerous times (Bai *et al.* 1986, Coupe *et al.* 1990, Pape *et al.* 1989, Hillgartner *et al.* 1996), and points to a primarily transcriptional control of the ACC gene by feed intake. Iritani (1992) found that ACC mRNA levels increased proportionately to the increase in intake of dietary carbohydrate in the rat. The close relationship between the amounts of grain intake and ACC mRNA level highlights the importance of this gene in carcass fat development.

Similar to ACC mRNA, fluctuations in the levels of LPL mRNA closely followed the nutritional status of the animals. Andersen *et al.* (1996) found LPL enzyme activities to be twice as high in sheep maintained on a high-energy diet (18.1 mJ/ day) when compared to animals maintained at a lower energy diet (11.1 mJ/day). This increase was consistent when animals were compared at a similar age or similar slaughter weight. Bas (1992) also

reported the close link between energy intake and LPL activity in goats. Unfortunately, neither studies measured mRNA levels, although the current study would suggest transcriptional control. It has been suggested that LPL is regulated transcriptionaly in response to nutritional status (Ladu *et al.* 1991), insulin (Ong *et al.*1988, Terrettazi *et al.* 1994) and cold exposure (Mitchell *et al.* 1992). Other studies have found that insulin regulation (Amri *et al.* 1996, Semenkovich *et al.* 1989) and fasting (Doolittle *et al.* 1990) resulting in changes in only LPL enzyme activity. The current study would support the theory that both ACC and LPL in ruminants are regulated primarily at the transcriptional level in response to nutritional status.

It is important to note that the changes seen in ACC and LPL mRNA levels in response to energy intake could be indirect. The changes in energy intake may be affecting another gene or set of genes that control the effect of the enzyme and/or hormone levels resulting in the change in ACC and LPL mRNA. Recent work surrounding insulin, the major hormone involved in energy metabolism, has produced conflicting results. Several authors suggest insulin's effect on ACC (Mabrouk *et al.* 1990) or LPL (Fried *et al.* 1990, Semenkovich *et al.* 1989, Raynolds *et al.* 1990, Amri *et al.* 1996, Simisolo *et al.* 1992) are entirely post-translational. Others suggest insulin affects ACC and LPL at the transcriptional level (Ong *et al.* 1988, Iritani 1992, Coupe *et al.* 1990, Girard *et al.* 1994). Indirect changes in mRNA levels for these genes would reduce their usefulness as candidate genes.

Changes in mRNA levels in response to feed intake could explain the sudden drop seen in month 7 for both ACC and LPL mRNA, as animals were subjected to an overnight

fast prior to slaughter. Another possibility for the reduction seen in month 7 for both ACC and LPL mRNA levels is found in the work of Haugebak *et al.* (1974) who found that LPL activity decreased in the adipose tissue of sheep as the duration in the feedlot increased. The authors concluded that this was primarily due to an increase in adipocyte size, and the reduction was eliminated when animals were compared on the basis of cell number.

It might seem possible that this lower value is the result of a reduced growth rate seen in animals as they near the plateau of their growth curve. However, Trenkle and Marple (1983) reviewed data showing that the late phase of the growth curve often results in a rapid acceleration of adipose tissue due to the diminishing energy requirements for muscle and skeletal growth.

iv. Relationships Between mRNA Level of the Genes and Growth and Tail Measurements

Positive and significant relationships between daily gains in tail thickness and circumference with adjusted and unadjusted ACC and LPL mRNA levels provided valuable evidence that these genes play pivotal roles in the deposition of fat. The findings also link the transcriptional product to the actual phenotype of the animal, pointing to a transcriptional control mechanism of LPL and ACC genes. If this is the case, mutations that cause even small changes in transcription rate could have drastic effects on carcass fat content. Recently, Jemma *et al.* (1996) found a link between polymorphisms in LPL and obesity in humans. Also, a single base pair substitution in the LPL gene has resulted in hypertriglyceridemia, obesity, hypertension, coronary heart disease and non-insulin-dependent diabetes (Tenkanen *et al.* 1994). Likewise, Cheema and Clandinin (1996) have found elevated ACC mRNA

levels in obese strains of mice.

Conventional methods of detecting genes involved in the regulation of fat metabolism include slaughtering different groups of individuals subjected to various nutritional treatments (Cheema and Clandinin 1986, Degail *et al.* 1992, Fried *et al.* 1991), or studying lines differing in body fat (Butler-Hogg and Johnsson 1986, Latif and Owen 1980). It should be emphasized that rate of change in tail dimensions (daily gains in the thickness and circumference of the tail), which reflected the temporal changes in rate of fat deposition in response to short-term variation in energy intake, is the fundamental advantage of this experiment over those reported previously. Such a measure of fat accumulation on live animals cannot be easily taken in any other breed or species, and has not been previously reported, particularly in ruminants. This unique measurement may provide information regarding subcutaneous and intermuscular fat depots, as tail fat content showed strong positive correlations with tail thickness and circumference ($r=0.84$ and $r=0.89$, respectively, Table 17), and weight of tail fat, in turn, showed strong correlations with subcutaneous and intermuscular fat in the carcass ($r=0.99$ and $r=0.95$, respectively). Therefore, levels of ACC and LPL mRNA in the tail adipocyte reflect the activities of these genes in subcutaneous and intermuscular fat as well.

Although regressions of daily gains in tail thickness and circumference on mRNA levels of ACC and LPL were statistically significant, partial R^2 values were small (5.8% to 8.1% for unadjusted and 3.9% to 6.2% for adjusted). When the rate of fat accumulation is regressed on mRNA of a single gene, small R^2 values are expected, because fat deposition

is controlled by a complex array of genes as well as many non-genetic factors. The small number of lambs used in this study could have contributed to the small R^2 values, since the differences in age at slaughter, sex, age of dam, etc., were not have been statistically removed. The findings, however, seemed quite logical and consistent, indicating that significant differences were not due to chance, despite the small number of animals. In addition, the observation that ACC and LPL mRNA were significantly related to the rate of fat accumulation, measured as the rate of gain in tail circumference and thickness, but failed to show any association with the actual measures of body weight and size of the tail, which have been accumulated over time, add supporting evidence that ACC and LPL influence rate of fat accumulation.

Unadjusted aP2 mRNA showed weak but positive relationships ($P<0.10$) with daily gains in tail thickness and circumference, suggesting that aP2 mRNA mirrored lipogenic activity within the fat depot. The lack of significant differences in mRNA levels among the time periods during growth implies that changes in energy intake had little effect on aP2 activity. This suggests that aP2 may not be transcriptionally controlled in response to feed intake. However, aP2 was the only gene that showed any association ($P<0.01$) with daily weight gain and tail length. Therefore, it could be concluded that while the level of aP2 mRNA in adipocytes is not affected by short-term changes in energy intake, it is positively associated with fat accumulation in fast-growing lambs. Since Karakul is an unimproved breed with a high potential for fat accumulation, it is expected that a considerable proportion of gain in body weight was the result of fat accumulation. The strong correlation between

slaughter weight and weight of fat in the tail (r=0.94, Table 17) implies that fast-growing, heavier Karakul lambs accumulated more fat, and such animals tended to have higher aP2 mRNA than the slow-growing animals with lower levels of body fat.

v. Ratio of mRNA of the Genes

There was a lack of strong relationships between various ratios of ACC, aP2, HSL, and LPL mRNA and the tail measurements. In the carcass composition trial, unadjusted LPL/ACC and adjusted LPL/ACC, and LPL/aP2 showed significant breed differences, and the ratios of mRNA of some of the genes showed stronger correlations with percentages of carcass tissues than did mRNA of a single gene. The difference between the results of these two trials was mainly the result of very low LPL mRNA in the Texel breed, indicating that differences among the breeds used in the first trial were more pronounced for the ratios of mRNA of the genes than among individuals within the Karakul breed.

vi. Cross-Product of mRNA of the Genes

With the exception of HSL*aP2, the cross-product of adjusted and unadjusted mRNA of the genes showed trends similar to ACC and LPL mRNA values, revealing the strong influence of ACC and LPL on the cross-products. Similarly, daily gains in tail circumference and thickness, which were accurate measures of short-term changes in the fat content of the tail, were positively and significantly related to almost all the cross-products of the genes. It could be argued that significant regressions on cross products were expected, as similar regressions existed for ACC, LPL, and aP2 alone. It is interesting to note, however, that stronger probability levels and higher partial R^2 values were seen in the case of cross

products compared with mRNA of each of the genes alone. The best equations explaining the variations in daily gains in tail thickness and circumference, judged by the magnitude of R^2, were ACC*aP2, followed closely by LPL*aP2, explaining between 10% to 12% of the variations in these two traits. The highest R^2 values for a single gene were 7.6% and 7.7% (for daily gains in tail thickness and tail circumference on LPL mRNA, Table 19), showing a 4-percentage increase in the explanatory power of the regression equations containing cross-products.

The interaction of the genes highlights the complexity of fat metabolism in the growing animal, and is in agreement with the findings of Sinnet-Smith and Woolliams (1987) who reported that monitoring all aspects of fat metabolism (fatty acid uptake, *de novo* synthesis, lipolysis) was more informative than studying each component separately. These findings may also imply that the likelihood of finding a useful DNA marker for carcass fat based on mutations in a single gene may not be a realistic objective, and a panel of markers based on mutations in several key enzymes controlling energy metabolism may be needed.

It is interesting to note that unadjusted LPL*aP2 best explained the variations in percentages of subcutaneous fat, intermuscular fat, total carcass fat, carcass muscle, and the ratios of muscle to all the measures of carcass fat in trial I (Table 13). The resemblance between the results of the two trials for LPL*aP2 indicate that these relationships are not coincidental, and these two genes interact with each other. Again, the strong relationship is best explained by the function of these genes. LPL plays an important role in the uptake of circulating triglyceride (Eckel 1989), and aP2 has been shown to be involved in the

trafficking of fatty acids within the cell (Matarese *et al.* 1989). The combination of these two genes could provide a more complete view of fatty acid storage within the cell. Animals that have higher LPL and aP2 levels are able to take up more circulating triglycerides (higher LPL) and also efficiently transport these fatty acids to storage droplets (higher aP2).

Similarly, the high explanatory power of unadjusted ACC*aP2 for the variations in daily gains in tail thickness and tail circumference can be explained by the function of these two genes, as ACC is the key regulatory enzyme involved in *de novo* synthesis of fatty acids (Vernon 1992). As was the case with LPL, the combination of LPL and aP2 could be providing a more complete view of fatty acid storage within the cell. Animals that are able to both synthesize more fatty acids (higher LPL) and efficiently transport these fatty acids (higher aP2) to fat droplets in the adipocyte accumulate more fat.

The finding that body weight was not related to any of the cross-products of mRNA of the genes, as was the case for individual mRNA, is expected since body weight is a trait accumulated over time, and consists of different tissues and organs whose growth rate are not directly controlled by the genes studied.

The relationships between actual measures of tail dimensions (thickness, length, circumference) and HSL*aP2 is difficult to interpret. The difficulty arises from the fact that the adjusted and unadjusted mRNA levels of HSL and aP2 genes, individually or their cross product, did not show significant variations across the growth periods. In addition, adjusted or unadjusted HSL mRNA was not related to any of the measures of body growth or tail dimensions. The adjusted and unadjusted aP2 mRNA levels, however, showed positive,

although weak, relationships with tail length (Tables 18 and 20). There is no information in the literature to compare with these results. Small partial R^2 values (less than 2%) indicate that the contribution of HSL*aP2 to total carcass fat is minor.

Since unadjusted ACC and LPL mRNA levels showed the strongest relationships with daily gains in tail thickness and circumference, it was anticipated that ACC*LPL would show the strongest relationship with these tail measurements as well. However, since both ACC and LPL reacted in a similar manner, it might be concluded that animals with higher LPL also had higher ACC. If this were the case, the interaction of the two genes would not provide additional information. Although the patterns of change in adjusted and unadjusted mRNA of genes alone and the cross-products of the genes over the growth periods were the same, and the regression coefficients of weight and tail measurements on adjusted and unadjusted mRNA values had similar signs, adjustment for β-actin reduced the explanatory powers of the regression equations when individual genes were considered individually or their cross-products were considered. Partial R^2 values for unadjusted mRNA levels of a single gene were 2% points higher than those for the adjusted mRNA levels (Table 19 and 21). Adjusted mRNA levels reduced the partial R^2 values of the cross-products of the genes by 0.6 to over 4% (Tables 24 and 26) as well.

vii. Summary

The tail of Karakul sheep provided an excellent model to monitor temporal changes in body fat in response to short-term changes in energy intake. Daily gains in tail circumference and thickness were accurate measures of the rate of fat accumulation and

mobilization on live lambs, while daily gain in tail length seemed to parallel the rate of skeletal growth, and was related to chronological age. Weight of tail after slaughter was, however, closely related to its length, as well as to its thickness and circumference. Fat comprised 92.5% of the tail mass, and showed high correlations (>0.94) with carcass subcutaneous and intermuscular fat, and was therefore a good estimator of carcass fat on the live lamb.

Close associations were observed between daily gains in tail circumference and thickness and mRNA levels of LPL and ACC in the Karakul lambs, and provided compelling evidence to suggest that LPL and ACC are key regulators of fat accumulation in the growing lamb. aP2 mRNA did not fluctuate as the plane of nutrition changed, but the data provided evidence that higher aP2 mRNA levels may be associated with a higher rate of weight gain and skeletal growth, and a gradual increase in body fat, resulting in a high amount of fat accumulation during the animal's life span.

The cross products of the genes mRNA levels explained a higher proportion of variation in the rate of fat accumulation than did mRNA levels of each gene alone. These findings showed that the genes interact with each other, and point to a biological system in which the sum of all components maybe more informative than each component taken separately.

6. CONCLUSIONS

Both the rate of fat accumulation in the tail of Karakul lambs in the growth trial and carcass fat measurements in the carcass composition trial showed positive and significant involvement of LPL. These relationships highlight the significant role that LPL plays in the fat metabolism (*via* uptake of circulating triglycerides) in sheep. Since LPL mRNA levels were significantly lower in lean Texel when compared to fatter Dorset, it may be concluded that LPL holds promise in the search for markers for carcass fat content in sheep. The lower level of LPL mRNA in the Texel breed might be the result of mutations, resulting in lower transcription rates and/or lower efficiency of hybridization of the probe with mRNA. These mutations may be in the promoter and/ or enhancer of the LPL gene, or in another gene, such as insulin, that influences LPL expression. Although mRNA levels of LPL in adipose tissues of a group of animals that are managed similarly can be used as a measure of carcass fatness, the methodology is too cumbersome and too expensive to be routinely used in animal breeding programs. An attempt should be made to develop DNA markers for the LPL gene.

The observation that LPL mRNA levels in Dorset were similar to that in the Texel-Dorset cross, but significantly higher than Texel, highlights the complexity of fat accumulation in the ruminant animal. Although LPL mRNA levels are high in the Texel cross, it still appears to accumulate more fat than the Texel, suggesting there are other genes affecting fat accumulation.

The close tie between the ACC mRNA levels and energy intake during growth, and positive and significant correlations between ACC mRNA levels and growth rate of the tail

of Karakul lambs are evidence that ACC plays a pivotal role in fat metabolism (*via* fatty acid synthesis) in growing lambs. ACC, however, may not be a proper candidate gene for the development of DNA markers for carcass quality traits, due its lack of association with carcass composition and absence of differences among breeds

The results suggested that HSL was not a good indicator of carcass fatness in sheep. Although aP2 did not show significant differences among breeds (carcass composition trial), nor did it change during growth periods (Karakul growth trial), its interaction with LPL and ACC would suggest its crucial role in fat accumulation in sheep. In particular, the findings that LPL*aP2 mRNA levels showed strong relationships, both with carcass characteristics and with growth of the fat-tail warrants further investigation. This could be considered as evidence for the interaction between genes at the cellular level. Perhaps high levels of LPL and ACC activities, which result in a high rate of uptake of circulating triglycerides and a high rate of fatty acid synthesis, respectively, require high levels of fatty acid trafficking within adipocytes, which is provided by the aP2 gene. Also, there was some evidence in Karakul growth trial that lambs with higher aP2 mRNA levels tended to accumulate more fat over their lifetime.

In both trials, cross-products of the gene's mRNA levels explained higher proportions of variation in the traits studied, compared with mRNA level of each gene alone. These observations also point out the presence of interactions between genes. It may be concluded that searching for a DNA marker in only one gene may not be a reasonable expectation, unless a point mutation drastically changes the polypeptide structure and either

impairs or improves the enzyme function and activity in a desirable direction.

Fat comprised 93% of the weight of the tail in Karakul lambs. Daily gains in thickness and circumference of the tail were accurate measures of rate fat accumulation in response to short-term fluctuations in energy intake in lambs. It was shown for the first time that changes in the length of the tail are associated with chronological age and the growth of skeleton, and are not influenced by short-term changes in energy intake. Likewise, very high correlations ($r>0.94$) between amount of fat in the tail and subcutaneous fat and total carcass fat indicates that the Karakul is a valuable model for assessing growth of carcass fat in live animals, and is an excellent model for simultaneous estimation of rate of fat deposition, level of gene expression, and enzyme activity.

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