Chapter 1 Introduction

The work presented in this thesis was part of a project entitled 'Biology of nutrient partitioning', funded by the Australian Sheep Industry CRC (Project 1.5.5). Optimising production requires knowledge of the biological variation between genetically different Merino sheep in the way that they direct ingested protein and energy to wool and meat and the mechanisms that are responsible for this variation. The overall objective of Project 1.5.5 was to obtain information of this type (Hatcher 2006).

Genetic improvement in the sheep industry is increasingly being made using selection indices that include contributions from many traits. These traits include clean fleece weight, fibre diameter, liveweight, disease resistance and reproductive performance; and the traits are weighted according to genetic parameters and economic value. It is not known if this multi-trait selection of sheep for wool and meat production, i.e. 'dual-purpose' sheep, leads to a 'trade off' between deposition of ingested protein and energy in the wool and body. It is also not known whether indices directed towards a primary selection goal (e.g. higher wool production) but with different combinations of secondary traits and weightings could generate genotypes that achieve their higher wool production by using different underlying gene combination pathways, and thereby different physiological and biological mechanisms. To answer these questions, appropriate experimental procedures needed to be established to enable rates of protein synthesis and degradation at the whole-body and individual tissue level and whole-body energy expenditure rate (heat production) to be determined.

Selection procedures currently used by many producers are based on genetic parameters estimated from industry data and implemented through Sheep Genetics Australia (the national sheep genetic evaluation program). Measures and derived estimated breeding values (EBVs) include wool growth rate, fibre diameter, liveweight, fatness and meat yield. Although these measures describe traits of economic significance for producers, they do not include the biological efficiency terms that also contribute to efficient use of feed resources and profit on farm. Therefore, another aim of the work described in this thesis was to find additional indicators of genetic superiority in individuals in flocks that could be used to achieve more rapid improvements in wool and meat production, and

thereby, increase profitability of the industry and improve the sustainability of environmental and social resources.

This thesis has eight chapters. Chapter 1 is this introduction and Chapter 2 is a review of literature that provides relevant background information concerning the physiological consequences of selecting Merino sheep for superior wool and meat production, and the protein and energy metabolism associated with differences in nutrient partitioning in ruminants. These chapters are followed by an experimental section of five chapters (Chapters 3 – 7), based on the studies reviewed in the literature review. Finally, the implications of the results are presented in an integrating discussion in Chapter 8.

Chapter 2 Literature Review

2.1 Physiological consequences of selecting Merino sheep for wool and meat production

How has genetic selection for wool and body growth changed dietary protein and energy utilisation in body tissues of individual animals? Do sheep, genetically selected for wool and muscle growth, exhibit 'metabolic wisdom' in the ways they regulate nutrient partitioning to skin and muscle? The answers to these and other related biological questions should assist in the understanding of the biological processes that are accessed and altered by selective breeding of sheep. In turn, these answers should provide clues to the adaptations that occur to support high rates of wool and meat production, and may suggest management strategies that enable improved genetic potential to be realised.

2.1.1 Selection for wool growth

For most strains of Australian Merinos, the major objective in selective breeding is attainment of high clean fleece weight, with attention to some additional traits of economic importance, most notably, average fibre diameter and body weight. This section examines the effects of selection directed primarily towards increasing clean fleece weight. The terms 'F+' and 'F-' have often been used in different studies to denote genetically high and low fleece-producing animals. It is obvious that selective breeding has generated a common desired output – superior wool growth – but the improvements in wool growth are most likely achieved by the actions of selection on different genes and thereby different physiological and biochemical mechanisms.

Effect of selection for wool growth on follicle and skin characteristics

Wool production by sheep is a function of the density, size and production of wool follicles in the skin (Williams 1987; Hynd and Masters 2002). Merinos selected for high fleece weight (F+) over a 35-year period produced 47 % more wool than sheep selected for low fleece weight (F-), and the higher wool production was supported by a 27 % greater follicle density and a 21 % greater ratio of secondary to primary follicles (Williams and Winston 1987). Sheep with higher ratios of secondary to primary skin

follicles produce finer wool than sheep with lower ratios (Hynd 1995), and the ratio is moderately heritable (the heritability for strong-wool, medium-wool and fine-wool Merinos is 0.3, 0.21 – 0.45 and 0.52, respectively). However, good nutrition during and immediately after lambing is critical to enable genetic potential to be achieved (Hynd 1995; Hynd *et al.* 1997).

The higher mass of follicular material and higher rate of cellular activity of the follicles in F+ sheep may also contribute to their high wool production (Williams 1987). Williams and Winston (1987) found follicle bulb diameter was identical in F+ and F- Merino lines. A more informative indication of fibre output is bulb volume combined with the turnover and migration rate of bulb cells. Williams and Winston (1987) reported F+ Merinos had a greater bulb cell volume and a higher proportion of mitotically active bulb cells than F- Merinos. However, they found no significant difference in the number of bulb cells per volume of bulb tissue between F+ and F-, and there has been no definitive demonstration, since that study, of any difference in the turnover rate of bulb cells between F+ and F- sheep.

There is some evidence that the structure and chemical composition of skins from high and low wool-producing sheep are different. Nay (1966) found F+ Merinos had deeper follicles that were embedded in a more organised vasculature. Williams and Winston (1987) reported F+ Merinos had deeper follicle bulbs (1.52 mm) than F- Merinos (1.38 mm). The skin of F+ Merinos was 19 % denser than F- sheep (Hales and Fawcett 1993), the skin from F+ Merino sheep was thicker and more fragile (Williams and Morley 1994), more wrinkly (Williams 1987), and had a lower skin collagen content (Williams and Morley 1994). The skin of F+ sheep had relatively more non-collagenous proteins, cystine and non-peptide bound α-amino nitrogen than that of F- sheep (Williams and Morley 1994). Masters *et al.* (2000) found that the concentration of hydroxyproline in skin tended to be lower in F+ sheep. Hydroxyproline is generated during the degradation of collagens (the major class of non-wool proteins in the skin) and represents about 13 % of the amino acid content of collagen (Berg and Kerr 1992). Thus, it is possible that low skin hydroxyproline concentration can be used as the basis for selecting sheep with a high potential wool growth.

The causes of differences in wool growth of sheep genetically selected for and against wool growth cannot be identified from the simple measures of skin and follicle characteristics alone (Williams 1987). Rather than relying on a single skin trait, such as

follicle size or density, it is better to investigate other possible physiological measures, such as protein synthesis rate in the epidermis, dermis and whole skin. Although technically difficult to determine, at present, skin protein turnover could potentially be used as a selection index to select and breed sheep with highly developed skin traits and eventually to facilitate the selection of superior animals at an earlier age.

Relationship between wool growth and skin protein metabolism

It has been proposed that genetic differences in wool growth are mediated partially via skin protein metabolism (Harris *et al.* 1993a; Adams *et al.* 2000b; Adams and Cronjé 2003). Adams and Cronjé (2003) suggested that genetic improvement in wool growth in Merino sheep is accompanied by a proportionate increase in total protein synthesis in the skin and proposed this may cause an increase in whole-body protein synthesis rate, given that skin protein synthesis contributes approximately 24 % of whole-body protein synthesis (24 % was calculated from data in Adams *et al.* 2000a). A high rate of protein synthesis requires a significant amount of energy input (Webster 1981; Reeds *et al.* 1985). Hatcher (2006) has suggested the main limitation in genotypes with a high potential for wool growth is energy rather than amino acids. Harris *et al.* (1994) confirmed that high protein synthesis in the skin imposes a higher energy cost compared with muscle. Therefore, the relative importance of energy metabolism in skin of sheep with different energy intakes needs to be further investigated.

Wool accounts for approximately 20 % of the protein synthesised in Merino sheep skin (Liu *et al.* 1998; Adams *et al.* 2000b), despite only 2 % to 3 % of the skin surface area being covered by fibre (calculated from data in Williams and Winston 1987 and Hales and Fawcett 1993); however, the proportion of skin protein synthesis that occurs within the follicle is unknown. Previous researchers have treated skin (including epidermis, dermis, follicles, fibre and sebaceous glands) as a single organ (Liu *et al.* 1998; Masters *et al.* 1999; Adams *et al.* 2000a). Adams *et al.* (2000b) reasoned that at least 50 % of the protein synthesis in sheep skin (12 % of whole-body protein synthesis) is probably associated with the wool follicle, but their conclusion was not based on direct measurements.

Treating skin as a homogenous organ carries a risk that the contribution of each part of the skin, epidermis and dermis, to wool growth will be overlooked. The epidermis is the outermost layer of skin that acts as a barrier preventing toxic chemicals and other

materials from penetrating deeper into the skin. It has been studied extensively in humans because of its clinical importance during scar recovery (Swanson and Melton 1997). In the case of Merino sheep, attention has been drawn to the epidermis in relation to its significance in the early stage of development of follicle populations and the number of cells in the follicle bulb (Hardy and Lyne 1956; Black 1987; Hardy 1992; Adelson *et al.* 2004). Migration of the epidermal melanocytes along the out-root sheath to the follicle bulb in relation to the development of pigmented wool fibres in adult Merino sheep was described by Forrest and Fleet (1986). The epidermis renews itself by division of stem cells at the dermal border at a rate that compensates for cells shed from the outer surface (Swanson and Melton 1997). The cells in the middle of the epidermis actively synthesise keratin to protect the surface. The blood supply to the skin consists of the epidermal capillaries that supply the blood to the epidermis; wool follicles are supplied with blood by the mid-dermal and dermal vascular networks (Nay 1966; Ryder 1968). If follicles account for at least 50 % of protein synthesis in the skin (although wool accounts for 20 % of total protein synthesis in the skin it must pass through the wool follicle) (Adams *et al.* 2000b), then the rest is distributed between the epidermis and the non-wool follicle components of the dermis. Both epidermis and dermis must maintain significant rates of protein synthesis to support wool growth and maintain the integrity of the skin; the relative contribution of non-follicular dermis and epidermis to skin protein synthesis is not known. If the dermis and epidermis could be separated and their protein synthesis rates estimated individually, it should be possible to gain further insights into the proportion of protein synthesis in skin which is associated with the wool follicle. An attempt to describe these processes quantitatively is made later in this thesis.

Effect of selection for wool growth on nutrient utilisation for wool growth

Nutritionally, feed intake appears to be regulated in an attempt to provide energy yielding nutrients and amino acids required by the animal's genetic potential (Ellis *et al.* 2000). A series of studies on the Trangie fleece weight selection flocks showed that single-trait selection for high fleece weight led to increased feed intake as well as improved efficiency (g clean wool/kg feed) of conversion of food to wool (Dolling and Moore 1960; Ahmed *et al.* 1963). Further study of ewes from the Trangie F+ and F- selection flocks indicated that F+ ewes consumed 12 % to 19 % more feed/$LW^{0.75}$, as well as being more efficient in utilising ingested feed for wool growth, than F- ewes

across a range of diets (Kahn 1996). Efficiency of nutrient utilisation for wool growth accounted for virtually all of the difference in wool growth between fleece weight selection flocks (Williams and Miller 1965; Williams and Winston 1965; Williams 1966). From these results, it seems clear that efficiency of utilisation of feed for wool growth is increased by selection for wool production.

Kahn (1996) found selection for wool growth was associated with a 9 % increase in production of microbial protein in the rumen (g microbial N/g N intake), despite similar N digestibility in the whole tract. Lush *et al.* (1991) demonstrated that, across a range of ME intakes (0.7, 0.9, 1.1 and 1.3 M), F+ sheep absorbed 20 – 55 % more α-amino N from the gut than did F- sheep at the same intake. Williams (1976) studied cysteine metabolism in sheep from the Trangie F+ and F- selection flocks and showed that F+ sheep had a lower sulphur concentration in their wool (3.0 % vs 3.7 %) than F- sheep. At the same feed intake (g/d), the entry rate of blood cysteine in the F+ sheep was about 20 % lower than in F- sheep, but the percentage recovery of [^{15}S]-cysteine in the wool was higher (50 % vs 40 %), indicating that these F+ sheep were more efficient in utilising absorbed cysteine for wool growth. The various studies cited above suggest that these F+ sheep produced more microbial amino acids, absorbed these amino acids more efficiently from the gut and made more efficient use of the absorbed amino acids for wool protein synthesis.

Nutrients are supplied to the skin and wool follicles from the blood stream and so Black and Reis (1979) and Williams (1987) reasoned wool production might be related to blood flow rate through the skin. Direct measurements of blood flow through the skin of Merino sheep showed that skin blood flow rates (ml/100 g skin tissue per min (Hocking Edwards and Hynd 1991) and ml/cm^2 skin per min (Hales and Fawcett 1993)) were significantly greater in the high wool producers than the low wool producers. On the other hand, studies on Romney sheep showed no relationship between blood flow per area skin (g/cm^2 per min) and wool production (Harris *et al.* 1993a), but these researchers suggested that the rate of uptake of nutrients, especially cysteine, by the cells of skin was greater in Romney sheep selected for high wool growth. These studies suggest the improvements in wool growth resulting from different types of genetic selection may be achieved via different physiological mechanisms.

Woolaston (1987) and Williams (1987) reported that wool growth of F+ Merino sheep responded more to increased nutrition than F- sheep. Critical questions regarding F+

sheep are: do these animals need a higher level of nutrition to maintain their superior wool production, and do they have a higher maintenance nutrition requirement that will cause them to suffer greater liveweight loss in periods of nutritional inadequacy. Answers to these questions will help farmers decide if it is worthwhile breeding sheep for higher wool growth potential, especially for use in regions that are prone to drought periods when it is not always possible to offer these animals an appropriate level of nutrition.

Effect of selection for wool growth on protein and energy partitioning between wool and meat

Based on evidence from single character selection lines, some workers (Hatcher 2006) suggested there is a 'trade off' between deposition of protein in meat and wool, such that improvement in wool production occurs at the expense of meat production and vice versa. If this is true, then, single-trait genetic selection specifically for wool growth might have created animals which partition more protein towards wool at the cost of reduced muscle protein deposition.

The most extreme genetic differences for nutrient utilisation for wool and muscle growth are found when two different breeds are compared. Graham and Searle (1982) investigated the partitioning of available protein and ME to wool and muscle in two breeds of sheep (10-wk-old Dorset Horn and Corriedale wether lambs weighing approximately 19 kg). Both breeds were offered rations of 700 g feed/d and 1000 g feed/d of a mixture of lucerne hay, maize, soybean meal and casein. The deposition of ingested energy and nitrogen in wool and body tissues was assessed. Those animals with the higher wool-growth potential (Corriedale sheep) maintained their wool-growth superiority at both feeding levels. To achieve a higher wool growth, the Corriedale sheep diverted more protein and ME away from body growth to wool production than Dorset Horn sheep. The deposition of both protein and fat in the body tissues was restricted in the animals with higher wool-growth potential. This effect was exacerbated at a lower level of nutrition, when the Corriedale sheep partitioned an even greater fraction of absorbed protein towards wool at the expense of body protein deposition (Corriedale: 57 % vs Dorset Horn: 43 %). These results highlighted the competition between wool and muscle for protein and available ME in these higher wool-producing sheep. No explanation was given about the physiological basis of the Corriedale's wool-growth superiority under different nutritional conditions. This breed difference in

nutrient utilisation for wool and muscle growth may not indicate what is possible by selection for traits, such as wool growth and meat production, within Merino sheep.

For example, Cronjé and Smuts (1994) studied protein and energy partitioning between wool and body tissues in 2-year-old Merino rams with high and low wool-growth potential. They found the high wool-producing sheep lost their wool-growth superiority relative to the low wool producers when they were fed at maintenance level. This situation is different to that reported in the study of Friend and Robards (2005) in which Merino sheep with a high potential for wool growth maintained their relative superiority in wool growth rate irrespective of dietary treatment (restricted or *ad libitum*). Cronjé and Smuts (1994) concluded that increased wool production is the result of greater partitioning of available nutrients to wool, and suggested that high wool-producing sheep maintained the priority for wool growth at the expense of other body functions. To explain why those high wool producers lost their wool-growth superiority on a maintenance diet, Cronjé and Smuts (1994) reasoned that high wool producers were able to adapt to a low nutritional feeding regime by decreasing wool growth rate. Williams and Morley (1994), using Merino sheep arising from Trangie lines selected for and against high wool growth over a number of generations, also found that sheep with an improved capacity for wool production were more sensitive in wool growth to alterations in nutrient supply.

In summary, selection for wool growth has generated animals with the ability to partition more available protein and energy towards wool growth, but with variable effects on the deposition of protein and energy in other tissues in the body. Partitioning of nutrients to different body tissues is affected by genetic selection, but the consequent changes that regulate the physiological and metabolic processes in tissues such as skin, follicle and muscle, are not yet known.

2.1.2 Selection for body growth

Herd *et al.* (1993) investigated whether divergent single-trait selection for weaning weight in Merino sheep affected the net efficiency of liveweight gain and wool growth. No differences in the net efficiency of feed use for liveweight gain or for wool growth were found. These researchers suggested that selection for increased weaning weight would not be an advantage in wool producing flocks; meat production per ha would be increased by only 9 % at the expense of a 24 % reduction in wool production. From a

profitability viewpoint, selection of animals for high weaning weight might not be as useful as selection for efficiency of meat and wool growth.

How selection for liveweight affects the efficiency of protein and total energy gain has been studied in mice. Bernier *et al.* (1987) found mice selected for increased post-weaning gain used ME 14 % more efficiently for protein deposition than control mice. Mice might not be unique in this regard. It is well known that the processes of protein metabolism are energy dependent (Pratt and Cornely 2004) and heat production is proportional to lean mass and protein synthesis (Webster 1981). Therefore, it might be possible to identify and select genotypes that differ in protein synthesis and associated heat production in order to reduce the amount of feed used to obtain a particular weight and body composition, i.e. to increase feed conversion efficiency (Oddy and Sainz 2002).

Changed partitioning of ingested protein between body tissue and wool as a consequence of genetic selection for weaning weight has also been identified. Davis (1987) reported that selecting sheep for higher weaning weight resulted in increased body growth with a proportional decrease in wool production per unit feed intake. Oddy *et al.* (1989) used those same sheep (selected for weaning weight) and found that faster growing lambs (W+) ate more feed and deposited more protein in body tissues and less in wool than slower growing ones (W-), but the ratio of whole-body protein retention to protein intake was identical in these two lines. Therefore, single-trait genetic selection for weaning weight in Merino sheep might have created animals which partition more protein towards muscle at the cost of reduced wool protein production.

Oddy (1993; 1999) argued that the change in partitioning of retained protein between body tissues and wool occurred because the W+ lambs degraded less whole-body and muscle protein than the W- lambs, rather than increasing whole-body or muscle protein synthesis rates. Protein degradation rate, therefore, might be a key consequence of genetic differences that result in changes in rates of muscle protein deposition (Oddy *et al.* 1995).

That liveweight differences can affect the protein synthesis rates in muscle and skin and wool growth has been illustrated by Liu *et al.* (1998). A study was made of two groups of 5-month-old Merino lambs differing in liveweight (33 kg vs 25 kg; the basis of the difference was not due to genetic selection, *S.M. Liu, Feb. 2007, personal*

communication). Both heavy and light sheep had similar rates of protein synthesis (%/d) in skin and in muscle at the same level of intake. Liveweight had no significant effect on the protein synthesis rate in muscle, which is in line with the conclusions drawn by Oddy *et al.* (1995). However, the heavier sheep grew more wool than the lighter sheep, irrespective of feeding level. The authors proposed that the heavier sheep had more muscle protein reserves, more skin and more follicles than the lighter ones. These results illustrate that transfer of protein from muscle to wool when feed is limited, and replenishment of muscle when surplus nutrition is supplied, may be an important mechanism in maintaining amino acid supply for wool production.

2.1.3 Multi-trait selection of sheep for both wool and meat production

The Australian Merino industry has been continuously challenged to produce more high quality wool and at the same time to increase meat production. Genetic selection in the sheep industry is increasingly being done using selection indices consisting of combinations of estimated breeding values (EBVs) (Brown *et al.* 2000). Selection indices contain contributions from many traits weighted according to both genetic parameters and economic value. An animal's EBVs are generated using measured information for a desired trait and other related traits from the animal itself and from relatives. The genetic parameters are used to estimate EBVs (MGS 2004). By using EBVs combined into suitable indices to inform selection decisions, it is possible to select animals that have superior genetic merit for several traits simultaneously, e.g. higher wool growth and liveweight, and lower fibre diameter.

There are no major genetic antagonisms between wool and meat traits (Safari *et al.* 2005) and simultaneous improvement of wool and meat production (dual-purpose) can be achieved by using appropriate selection indices (Fogarty *et al.* 2006). However, quantitative genetics alone cannot predict how selection pressure affects 'fitness' of the dual-purpose Merino over the long-term. Moreover, without knowledge of feed intake, it is not clear if there is an increased *efficiency* of ingested protein and energy utilisation for wool production and body tissue growth in animals selected for both high wool and liveweight, or if these animals simply consume more feed.

Studies conducted by Adams' research team at CSIRO, Perth (Adams and Cronjé 2003; Adams *et al.* 2005; Adams *et al.* 2006a; Adams *et al.* 2006b) suggested that selection for high fleece weight may not be desirable. These researchers proposed this was

because Merinos selected for higher fleece weight had lower body condition and energy stores (20 % less whole-body fat), and higher energy requirements than low fleece producers at the same liveweight. These researchers also suggested that lower energy reserves in high wool-producing sheep might contribute to lower conception rates and lower birth weights, especially for sheep on low energy diets, or in a variable climate.

Amino acids and peptides are used to synthesise proteins in different tissues and organs through different pathways (Figure 2.1). Muscle tissue and wool are both high in protein; from a nutritional point of view, it has been argued that muscle 'competes' with wool (skin) for amino acids to synthesise proteins in order to meet their respective physiological roles, especially in animals genetically selected for high wool growth (Cronjé and Smuts 1994; Adams *et al.* 2000b). The 'competition' argument is a useful start point from which to explore the feasibility of genetic selection for both wool and meat production. However, the mechanisms underlying the difference in wool and muscle production in selected Merino sheep are far more complex than the 'competition' theory suggests.

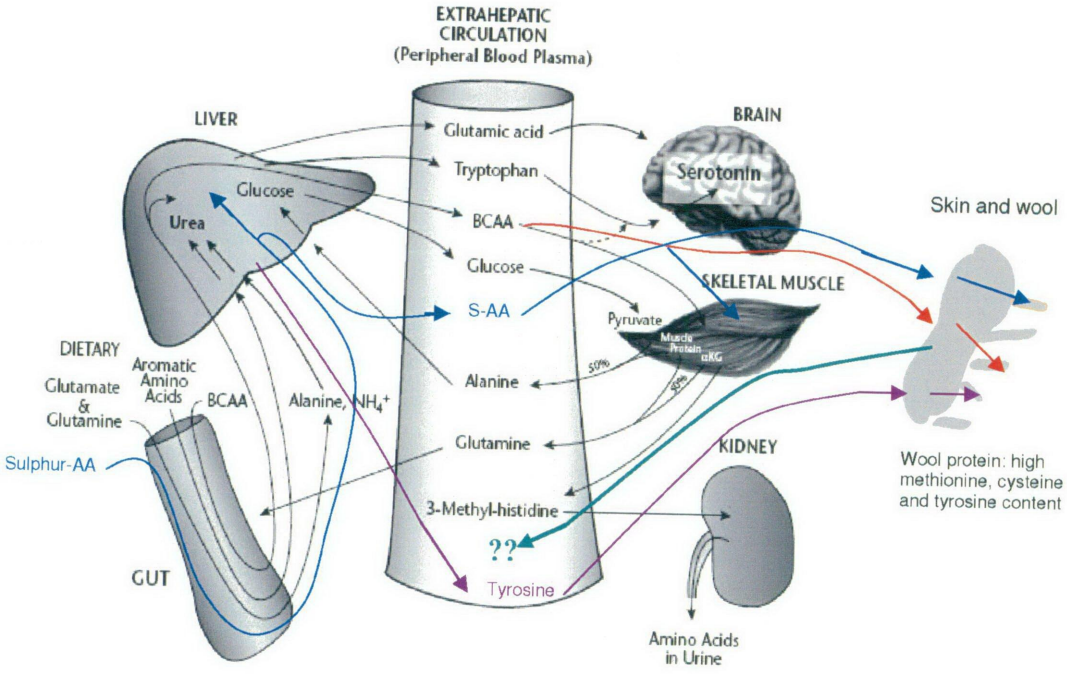

Figure 2.1 Inter-organ and tissue flows of amino acids and associations of amino acid metabolism (adapted from Young 1991 with minor changes)

2.1.4 Conclusion

This part of the review has been focused firstly on the physiological consequences of various single-trait selections for superior wool production (Williams 1987) or body growth (Oddy *et al.* 1998) in Merino sheep, and secondly on a number of questions raised about the feasibility of multi-trait selection.

Single-trait selection in Merino sheep for increased wool production has produced improvements in the gross efficiency of nutrient utilisation, microbial N production, uptake of amino acids by skin, and the capacity of follicles to use amino acids (protein synthesis and degradation). Studies reviewed in this section also show that single-trait selection for wool growth has generated animals with the ability to partition more available protein and energy toward wool growth, but has not apparently resulted in any improvement in the deposition of protein in the body. On the other hand, single-trait genetic selection for body growth has altered protein metabolism at the whole-body level, as well as at the muscle tissue level. A lower protein degradation rate plays a key role in the increase in muscle deposition observed in sheep selected for weaning weight. It appears that in superior genotypes, superiority is achieved by different combinations of genes and their gene-directed physiological processes, but the physiological and control mechanisms responsible have not been identified.

Currently, genetic improvement in the sheep industry is made using selection indices that contain contributions from many traits weighted according to genetic parameters and economic value (Fogarty *et al.* 2006; van der Werf 2006). It is not known if practical multi-trait selection of sheep leads to a 'trade off' in potential for wool or meat production or in other important attributes, such as resistance to disease and parasites. Furthermore, the information on the extent to which wool growth and meat production are constrained by the amount of protein absorbed from the gut to be partitioned among muscle, skin and wool is lacking. By developing a quantitative understanding of fluxes through key pathways, changes in physiology and ultimately control mechanisms responsible for protein and energy partitioning, we will be able to minimise the 'tradeoffs', if indeed they exist, and simultaneously improve both wool and meat turnoff in an informed way.

2.2 Protein and energy metabolism – regulation of nutrient partitioning in ruminants

Metabolism of protein and energy are closely interrelated (Gill *et al.* 1989; Pratt and Cornely 2004) and are mediated by genes and their interactions with many factors including nutrition, physiological status and hormones (Grizard *et al.* 1988; Douglas *et al.* 1991; Oddy 1993; Lobley 1994; Oddy and Sainz 2002; Adams *et al.* 2004; Chilliard *et al.* 2005). Knowledge of the aspects of protein and energy metabolism and factors affecting these two processes is pertinent to a better understanding of the regulation of nutrient partitioning between skin, wool and muscle in sheep.

2.2.1 Basic aspects of protein and energy supply to the animal

The protein and energy supply to tissues in ruminants is a function of events occurring in the rumen and subsequent intestinal digestion. The nature of ruminal microbial protein and energy metabolism is complex and has been reviewed elsewhere (Cotta and Hespell 1986; Wallace 1988; Broderick *et al.* 1991; Firkins 1996; Morrison 2000; Annison *et al.* 2002; Hristov *et al.* 2005; Valkeners *et al.* 2006).

The rumen, with its populations of bacteria, protozoa and fungi, enables the ruminant to digest dietary fibre that is not sufficiently digestible to be of value to monogastrics. The anaerobic fermentation of dietary carbohydrates by the microbial population in the rumen produces volatile fatty acids (VFAs), methane and carbon dioxide as end products. Energy generated by these processes is conserved in adenosine triphosphate (ATP) and is used for maintenance and growth of rumen microbes (Figure 2.2). Volatile fatty acids produced in the rumen are absorbed into blood circulation and distributed to the tissues where they undergo oxidation or are used for synthesis of lipids and/or glucose (Annison *et al.* 2002; McDonald *et al.* 2002).

Volatile fatty acids are the main source of energy absorbed by the ruminant, providing about 50 – 70 % of digestible energy intake in sheep and cows at approximately maintenance (Sutton 1971; 1979; 1985). Microbial cell organic matter (amino acids, lipids and polysaccharides) is another important energy source, which accounts for between 20 – 25 % of energy supply (Nolan and Dobos 2005). Since the dietary carbohydrates are fermented, ruminants normally absorb negligible amounts of sugars

such as glucose (Bannink and Tamminga 2005), and their glucose needs are met by gluconeogenesis, even in the fed state (Bergman *et al.* 1970; Lomax and Baird 1983).

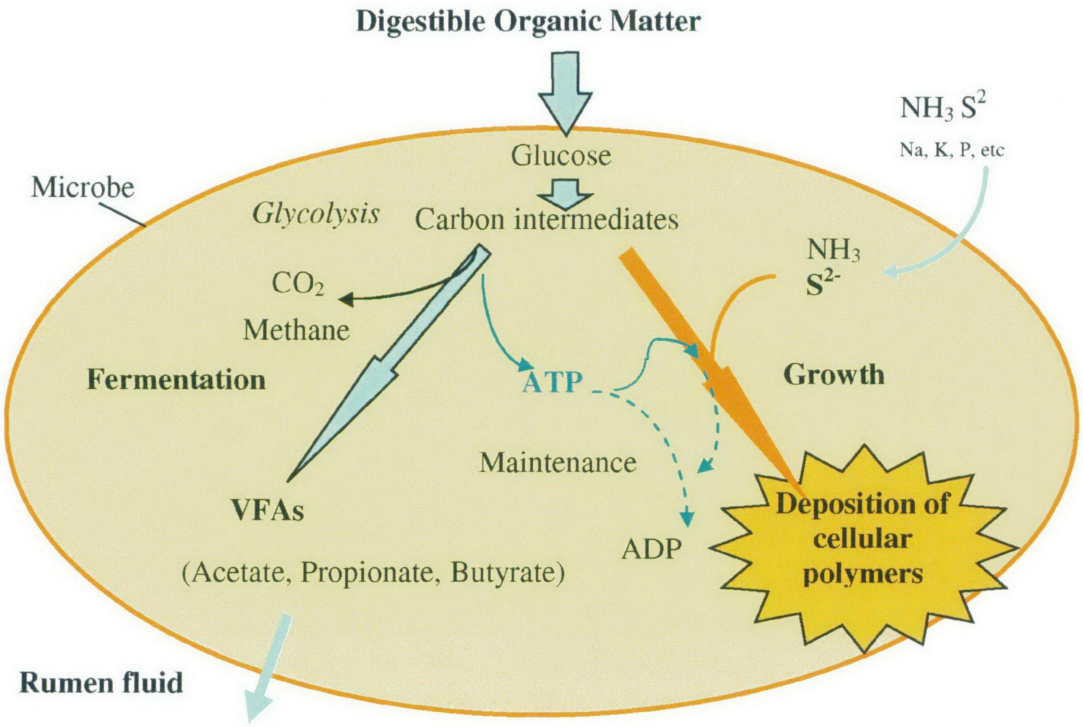

Figure 2.2 A schematic representation of the major pathways of carbohydrate metabolism in the rumen (adapted from Annison *et al.* 2002)

One of the prominent features characterising ruminants is the nitrogen metabolism in the rumen and the post-ruminal digestion of microbial or dietary 'escape' protein (Figure 2.3).

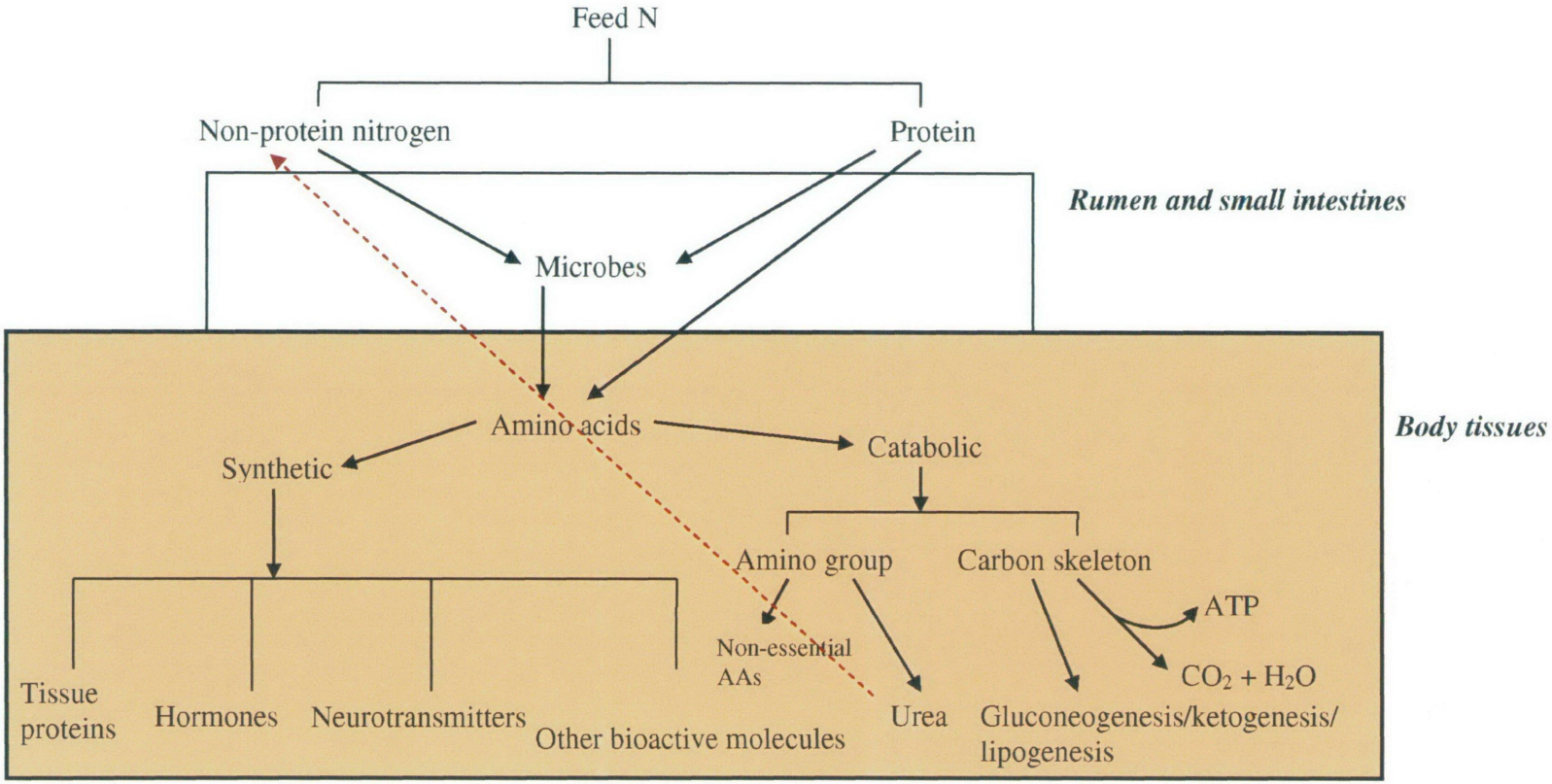

Figure 2.3 Simplified pathways in protein metabolism in ruminants (adapted from Atasoglu and Wallace 2003; D'Mello 2003)

Amino acids and peptides absorbed from the digestive tract of ruminants are of dietary, rumen microbial and endogenous origin. The majority of dietary protein (more than 80 %) is degraded in the rumen to yield peptides and amino acids, and ultimately VFAs (from the carbon chain) and ammonia if they are not used for microbial protein synthesis (Nolan and Dobos 2005). The amount of undegraded dietary protein entering the proximal duodenum depends on the total protein intake and the degree to which dietary protein is degraded in the rumen (Kristensen *et al.* 1982; Djouvinov and Todorov 1994). Microbial N is the major contributor to the total non-ammonia nitrogen flowing from the rumen to the proximal duodenum. For example, microbial N comprised about 60 % of the total N outflow in sheep on a high protein diet (Nolan *et al.* 1975), and 96 % with a low protein diet (Leibholz 1972). The contribution of endogenous protein is small relative to the other two sources, probably 0.4 – 2.5 g N/d in sheep offered a 11.4 – 34.3 g N/d lucerne-based diet (Neutze *et al.* 1997). However, recent work has shown that endogenous secretion might reach 20 % of the duodenal flow in lactating cows (Ouellet *et al.* 2002)

The availability of amino acids for tissue accretion and wool growth depends, in part, on the amino acid composition of the ruminal microorganisms. However, the amino acid composition of microbial protein available to sheep is relatively constant (Hogan *et al.* 1970; Storm and Ørskov 1983). Therefore, increasing the yield of microbial protein proportionally increases the availability of all amino acids. Wool growth is responsive to additional amino acids and to additional S amino acids in particular (Reis 1979; Reis *et al.* 1990).

2.2.2 Energy metabolism and expenditure in ruminants

Energy-producing and energy-requiring processes

Energy is ingested by animals and continuously released by their metabolic reactions and used to do work in living animals. Under normal conditions, energy is made available from oxidation of the three main groups of nutrients: carbohydrates, fats and proteins. Carbohydrates are an important group of energy yielding nutrients, which include sugars (mono and polysaccharides) and volatile fatty acids absorbed following digestion of carbohydrates in the rumen. Glycogen is the major form of carbohydrate storage in animals. Glycogen serves as a rapidly available and utilisable fuel for nearly all tissues of the body. When required for cellular processes, glycogen stored in the liver

and muscle breaks down to glucose, which is then oxidised to CO_2 and H_2O, whilst liberating energy predominantly as ATP. The majority of cells in the body are able to oxidise fats as a source of energy. Proteins of feed and microbial origin are hydrolysed to peptides and amino acids in the intestine, where they are absorbed and used in body tissues to synthesise proteins or are oxidised. Amino acids not used for synthesis are deaminated; the amino group is released and the corresponding keto acids are formed. These can be used for glucose synthesis or lipogenesis or oxidised to provide energy (Annison *et al.* 2002; McDonald *et al.* 2002).

Metabolisable energy (ME) is initially used for maintenance and physical processes, such as activity, digestion and absorption of nutrients from the intestinal tract, blood circulation and ion transport across cell membranes. Most of ME (about 80 % for rapidly growing non-lactating ruminants and about 60 % for lactating ruminants) is given off as heat, and only a small proportion is retained as animal products (Corbett *et al.* 1982; Kirchgeßner *et al.* 1982). When ME intake is above maintenance level, the energy liberated from oxidation of nutrients is used for synthesis of carbohydrate (glycogen in the liver and in muscle), fat (in adipose tissue) and protein (in muscle, enzymes, hormones and wool fibre). The energy cost of tissue deposition can be divided into two parts: the energy stored and the energy used in the synthesis of tissue components. In theory, the minimum cost of synthesising 1 g protein is about 4.5 kJ/g protein. This cost includes the cost of associated processes, i.e. the synthesis of messenger RNA, the cost of amino acid transport into cells, and the cost of peptide bond synthesis (Waterlow 2006). The energy cost of depositing 1 g protein, on the other hand, is about 20 kJ/g protein (Fuller *et al.* 1987). The large difference between the energy cost of deposition of 1 g protein and the theoretical cost of synthesising 1 g protein is the additional cost associated with protein degradation and turnover. Protein deposition is the net difference between protein synthesis and degradation. Protein degradation also requires energy. For instance, ATP hydrolysis is required in the process of ubiquitin-proteasome-dependent proteolysis (Attaix *et al.* 2002). However, protein accretion requires many cycles of synthesis and degradation, during which cellular structures are rebuilt, errors in misfolding of proteins are corrected and temporal requirements in enzyme activity are accounted for. Protein synthesis rates often exceed protein accretion rates by between 4- and 6-fold in normal growth (Houlihan *et al.*

1995). Accordingly, the energy cost of protein accretion far exceeds that of protein synthesis (Oddy and Sainz 2002).

It has been suggested that about 10 % of cellular energy requirements are related to proteolysis (Lobley 1994). Between 15 % and 22 % of total energy is expended in maintaining the processes of protein turnover (Reeds *et al.* 1985). The high-energy cost of protein synthesis and the entrainment of protein synthesis and degradation is the reason why the rate of protein turnover is strongly correlated with energy metabolism (Webster 1981).

Energy expenditure (heat production) and the quantification methods

The fate of energy ingested by a ruminant is illustrated in Figure 2.4. There are two major sources of energy loss during the digestive process: the loss associated with excreta and combustible gases, and that with the heat produced as a result of chemical and physical processes associated with digestion and metabolism (Agnew and Yan 2005).

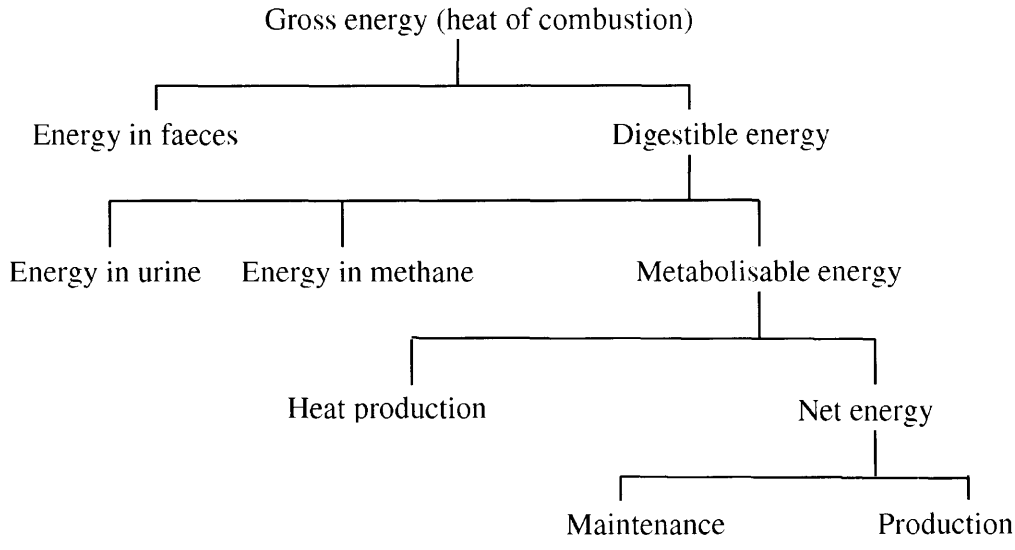

Figure 2.4 The partitioning of feed energy in the ruminant (adapted from McDonald *et al.* 2002)

Energy expenditure in fed animals can be estimated by directly measuring heat production, or by indirect calorimetry from respiratory CO_2 production and oxygen consumption obtained from sheep in respiratory chambers, or tracer-related CO_2 entry

rate and doubly-labelled water methods (Blaxter 1967; McDonald *et al.* 2002; Wolfe and Chinkes 2005a; Wolfe and Chinkes 2005b). The principles, assumptions and sources of possible errors are summarised in Table 2.1.

Indirect calorimetry is possible because energy transformation is governed by the laws of thermodynamics – the law of conservation of energy states that 'energy can not be created or destroyed, only changed in form' and Hess's law of constant heat summation states that 'the heat released by a chain reaction is independent of the chemical pathways, and dependent only on the end products' (Blaxter 1989). According to these laws, heat evolved in the enormously complex set of biochemical reactions that occurs in the body is the same as that which is measured when the same food is converted to the same end products by simple combustion in a calorimeter (Agnew and Yan 2005).

Tracer-related techniques, such as CO_2 entry rate using bicarbonate labelled with the stable isotope ^{13}C or doubly-labelled water ($^{2}H_2{}^{18}O$), are very attractive alternatives to experiments in pens or slaughter trials (indirect calorimetry). To balance the theoretical scientific study and the 'real world' demand from farmers, tracer techniques can also be used in free-living animals, permitting measurement of the influences of grazing, locomotion and weather, which may have significant effects on the energy metabolism of the animals. Apart from the theoretical limitations (Table 2.1), another concern about tracer techniques is their cost. However, improvements in the precision of mass spectrometers now make it feasible to use smaller doses of stable isotope tracers in large farm animals.

Table 2.1 Summary of methods of measuring whole-body heat production (whole-body energy expenditure rate, WBEE)

Method	Principles	Assumptions	Possible errors	Reference
Direct calorimetry	1. Same principle as Bomb Calorimeter 2. With estimates of heat production	1. Energy retention is constant during study 2. Energy available from feed accurately measured over course of the study	1. Measurement of initial value	Blaxter (1967)
Indirect calorimetry	1. Calculation of net rates of carbohydrate, fat and protein oxidation 2. WBEE calculation is based on estimates of CO_2 production (V_{CO2}) and O_2 (V_{O2})use during respiration and the stoichiometry of the oxidation of glucose, fatty acids and amino acids WBEE (kcal/d) = 3.9 V_{CO2} (L/d) /RQ + 1.11 V_{CO2} (L/d)	1. Excreted N is a final end-product of amino acid oxidation 2. Methane production represents a small heat loss, it can be measured and accounted for.	1. Respiratory quotient (RQ) of protein oxidation 2. Influence of other metabolic processes 3. Amino acid oxidation is based on the excretion rate of N	Wolfe and Chinkes (2005b)
CO_2 entry rate: bicarbonate labelled with the stable isotope ^{13}C	1. In the single-pool model, estimate of entry rate (Rco_2) is based on the rate of dilution of labelled bicarbonate by unlabelled bicarbonate in mixed blood 2. WBEE (MJ/d) = 0.0096 × Rco_2 (L/d) + 2.41 (Corbett *et al.* 1971)	1. RQ value is assumed and O_2 use is calculated from Rco_2 2. HCO_3 is the single, well mixed compartment through which all respiratory CO_2 passes before elimination from the body 3. The sequestering of ^{13}C in tissues is negligible during course of measurement	1. Assumed RQ 2. Incomplete oxidation of organic matter by the host animal 3. CO_2 released from the rumen 4. Possible changes in background enrichment during the period of sampling 5. $^{13}CO_2$ retention 6. Errors in calculation of isotope dose	Wolfe and Chinkes (2005b)
Doubly-labelled water ($^2H_2{}^{18}O$)	1. In the single-pool model the rate of loss (R_{CO2}) from the pool is equal to the fractional turnover rate × pool size 2. WBEE (KJ/d) = 16.32 R_{CO2} (L/d) /RQ + 4.58 R_{CO2} (L/d)	1. RQ value is assumed 2. Body water is a single compartment 3. Deuterium is lost only as H_2O, ^{18}O is lost both as H_2O and as CO_2 and the exchange between H_2O and CO_2 is rapid	1. Assumed RQ 2. Incorrect estimation of ^2H and ^{18}O fractionation 3. Altered initial volume of distribution of isotopes change in H_2O or CO_2 flux during sampling interval 4. Errors in calculation of isotope dose 5. The loss of H as methane in ruminants	Wolfe and Chinkes (2005a)

After investigating the costs of purchasing and analysing different tracers and the availability of analytical facilities (stable isotope gas ratio mass spectrometer) (Table 2.2), we chose the CO_2 entry rate technique to measure the heat production in the experiment described in Chapter 4.

Table 2.2 Costs of purchasing and analysing stable isotopes in Australia

Tracer type	Price (AU$/g)	Isotope analysis service	Cost of analysis ($/sample × No. of samples)
$NaH^{13}CO_3$	80 (supplied by Spectra Stable Isotopes, USA)	UNE, Armidale, NSW	15 × 200
$^2H_2{}^{18}O$	1010 (supplied by Sigma-Aldrich Co., USA)	CSIRO, Adelaide, SA	99 × 200

Bicarbonate labelled with the stable isotope ^{13}C has been used to estimate energy expenditure in sheep with acceptable accuracy (Sahlu *et al.* 1992; Rocha *et al.* 1994; Shew *et al.* 2000). The estimate of entry rate (Rco_2, also called 'net flux rate' or 'irreversible loss rate') is based on the rate of dilution of labelled ^{13}C-bicarbonate by the entry of unlabelled bicarbonate in mixed blood. Tracers are injected or infused via the jugular vein and mixed venous blood samples are taken from the right atrium of the heart or from other sites including the contra-lateral jugular vein. In some studies, urine, saliva or expired gases have also been sampled to estimate entry rate (White and Leng 1968; Sahlu *et al.* 1988). In single injection experiments, entry rate is derived from the dose injected divided by the area under the tracer / (tracer + tracee) curve versus time after the injection. In continuous infusion experiments, net flux is derived from the rate of tracer infusion divided by the plateau value of the tracer / (tracer + tracee) curve (Nolan and Leng 1974).

The equation chosen to calculate whole-body energy expenditure rate (WBEE, MJ/d) was as follows:

WBEE (MJ/d) = 0.0096 × Rco_2 (L/d) + 2.41 (Corbett *et al.* 1971).

The accuracy of the prediction of WBEE depends on the assumptions listed in Table 2.1. However, many of the errors related to the measurement of CO_2 entry rate were

discussed and accounted for by Corbett *et al.* (1971) when they developed the relationship between CO_2 entry rate and energy expenditure.

2.2.3 Specific aspects of protein synthesis and degradation in ruminants

The processes of protein synthesis and degradation

At any given moment, a typical cell contains thousands of different proteins. These must be synthesised in response to the cell's current needs, transported to the appropriate cellular location and ultimately degraded (Lehninger *et al.* 1993). The complexity of the mechanism for protein synthesis is illustrated in Figure 2.5.

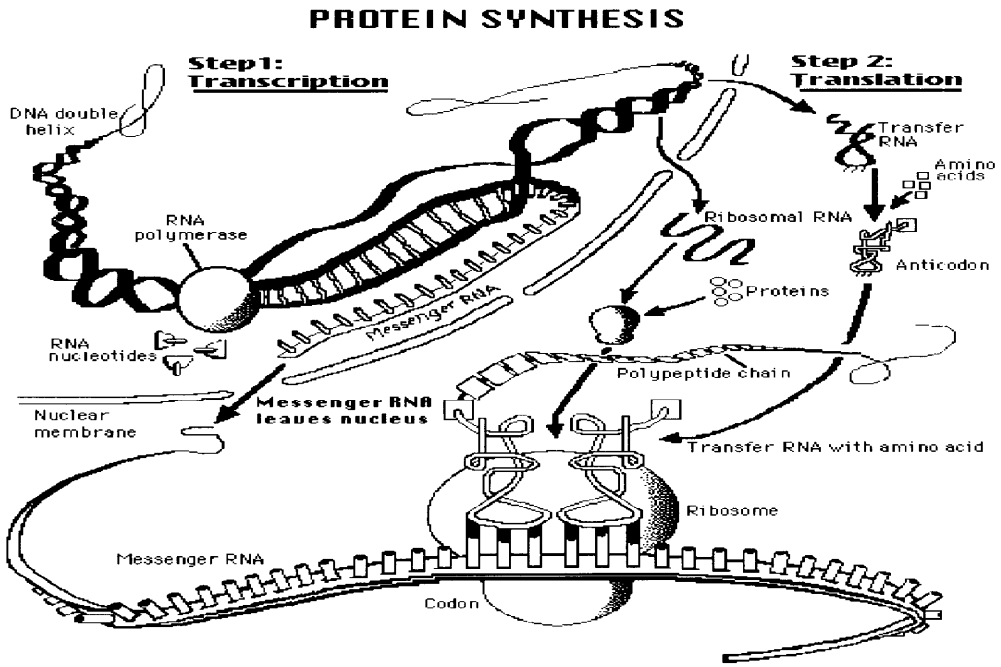

Figure 2.5 The illustration of the process of protein synthesis (reproduced from Farabee 2001)

Proteins are synthesised in the cells through the action of deoxyribonucleic acid (DNA), messenger ribonucleic acid (mRNA), ribosomal RNA and transfer RNA. DNA base sequences provide the genetic code or set of instructions for the RNA to collect amino acids within the cytoplasm of a cell (at the ribosomes) and link them together to form a protein chain (Arnstein and Cox 1992). Each sequence of three bases (from adenine, guanine, cytosine and thymine) is known as a codon, which almost uniquely specifies

an amino acid during translation of mRNA into protein. Adjacent codons on the DNA string determine the sequence in which the amino acids are linked by peptide bonds on the ribosome. Special codons act as 'punctuation' marks that are responsible for the termination of the sequence and the formation of the protein (Lehninger *et al.* 1993). At this point, the protein is far from complete; additional modifications may occur to newly synthesised proteins, including extensive relocation within the cell, proteolytic processing and the attachment of carbohydrate, lipid or other groups (Pratt and Cornely 2004).

The process of protein synthesis is relatively quick. An average human synthesises around 5085 protein molecules per second (Pirlet and Arthur-Goettig 1999). About 30 % of newly synthesised protein molecules are defective due to inaccurate translation or post-translational misfolding (Pratt and Cornely 2004). Even proteins that are normal when first synthesised may not be needed at the time when they are produced, perhaps due to lag between transcription, translation, relocation and functional requirements (Lehninger *et al.* 1993). These proteins will be selectively degraded within the cell at widely different rates (Lehninger *et al.* 1993). In this respect, the ubiquitin-proteasome-dependent pathway is the most common protein-degradation mechanism identified (Attaix 2001; Taillandier *et al.* 2004). However, currently little is known of mechanisms regulating the link between protein synthesis and the recycling of amino acids through pathways associated with protein degradation. It seems likely that selectivity of protein degradation, associated with re-utilisation of the amino acids derived from degradation, assists in conservation of essential amino acids (Waterlow *et al.* 1978).

Protein turnover is a general term that covers both synthesis and degradation of proteins (Rathmacher 2000). Protein turnover is essential for life since it provides the necessary flexibility that permits metabolic regulation and adaptation (Schimke 1977), while maintaining high integrity of translation from DNA to RNA to protein sequence. Animals benefit from the process of protein turnover. First of all, specific degradation of functionally defective, old, damaged or denatured protein molecules forces the selection of structurally superior proteins, and without selection at the level of the proteins, evolution would be impossible (Pirlet and Arthur-Goettig 1999; Pirlet 2003). Advantages conferred by the process of protein turnover summarised by Lobley (1993b) include:

1. The remodelling of organ and tissue structures enables animals to adapt to physiological and physical variation which is caused by growth, pregnancy and lactation.

2. The continuing mobilisation rapidly directs amino acids towards specific tasks, e.g. anti-infection needs, or to ensure the more vital organs such as brain, liver and kidneys, function properly at the expense of less vital tissues, especially skeletal muscle, during periods of food restriction.

3. Protein synthesis and degradation require energy, and heat is liberated. Between 15 % and 22 % of ME intake is expended in maintaining these processes (Reeds *et al.* 1985).

Quantification of protein synthesis and degradation

Understanding the quantitative aspects of protein turnover has been a major aim for nutritionists over the past three decades. The accretion or loss of body protein is the net result of the difference in the amounts of protein synthesised and degraded per unit time. From a growth point of view, rapid rates of protein accretion are supported by high rates of protein turnover (Riis 1983). For example, approximately 18 % of protein in muscle is broken down each day in the one-wk-old lamb, but this rate slows down with age to 3 % /d in 10-wk-old lambs (Figure 2.6).

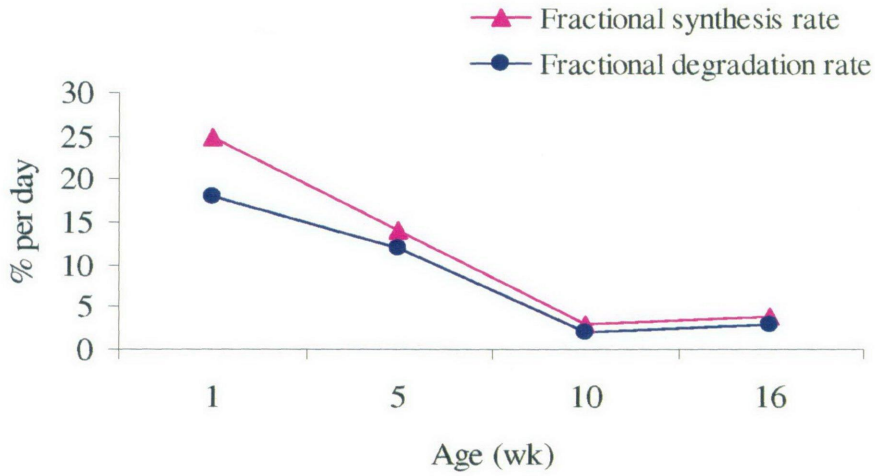

Figure 2.6 Fractional synthesis and degradation rate in muscle as a function of age in lambs (reproduced from Riis 1983)

The rate of protein synthesis greatly exceeds the rate of protein accretion (Table 2.3). The same accretion of protein could be reached either by an increase in protein synthesis or by a decrease in degradation (Garlick 1980). Therefore, direct measurement of protein synthesis and degradation in tissues can provide a better understanding of the processes that influence tissue protein gain and loss in sheep.

Table 2.3 Net growth of total body proteins and fractional rate of tissue protein synthesis (%/d) in lambs as they age (reproduced from Riis 1983)

Age	Net growth of total body proteins (%/d)	Fractional rate of tissue protein synthesis (%/d)	
		Skin	Muscles
1 wk	5.5	34	25
5 wks	1.6	15	14
10 wks	0.7	11	3
16 wks	0.5	18	4

Direct measurement of protein synthesis has been mostly conducted using isotopically-labelled amino acids or proteins; protein degradation is estimated concomitantly as the difference between protein synthesis and protein deposition. A summary of methods developed for measuring protein turnover and the assumptions and possible errors of the different methods is given in the following section.

Methods used for quantifying protein turnover

Most of the early studies on protein turnover were conducted at the whole-body level. End-product and precursor loss methods have been developed to investigate protein metabolism under different dietary conditions at the whole-body level in farm animals. These methods only provide averaged protein turnover rates for the individual organs and tissues. Subsequently, other techniques were developed to determine protein kinetics in different tissues. The following review will be focused on two methods: arterio-venous balance tracer method and tracer incorporation method.

The principal assumptions and possible errors of the different approaches developed for measuring protein turnover are summarised in Table 2.4.

Table 2.4 Summary of assumptions and limitations related to the methods used to measure protein turnover

Method	Essential assumptions	Possible errors	Reference
Whole-body protein turnover: 1. End-product method using continuous infusion or a single oral dose of ^{15}N-glycine or ^{15}N-protein 2. $^{13}CO_2$ end-product method using continuous infusion of ^{13}C-leucine 3. Precursor method using continuous infusion of ^{13}C-leucine or L-[ring $^{2}H_5$]-phenylalanine	1. The metabolic pool is in a 'steady state' and homogeneous 2. There is no recycling of label via protein breakdown to the free amino acid pool over the period of measurement 3. The metabolism of the tracer reflects that of the total amino acids in the body 4. The synthesis rate equals flux minus excretion; other metabolic pathways have no quantitative importance	1. Lack of homogeneity of the precursor and protein 'pool'. 2. The choice of tracer, tracer administration route and dose method 3. Sampling site 4. Amino acid recycling, i.e. assumption No. 2 does not hold	Matthews *et al.* (1980); Fern *et al.* (1981); Clarke and Bier (1982); Thompson *et al.* (1989b); Waterlow (2006)
Specific tissue protein turnover: tracer incorporation methods: 1. Constant infusion of [1-^{13}C]leucine 2. Flooding dose- L-[ring-d_5]phenylalanine	1. An isotopic 'steady state' exists 2. None of the tracer recycles during the course of incorporation 2. The enrichment of the immediate precursor is similar to that of the free amino acid in either blood or tissue homogenate	1. Constant infusion: recycling of tracer in tissues with high turnover rate ; Proteins exported during the infusion period are not included in the FSR 2. Flooding dose: large dose of amino acids may directly alter protein synthesis; tissue uptake of amino acids may be influenced by the flooding dose; high amino acid concentration may alter hormone balance	Lobley *et al.* (1992); Liu *et al.* (1998); Adams *et al.* (2000a)
Specific tissue protein turnover: arterio-venous balance tracer method 1. Constant infusion of continuous infusion of ^{13}C-leucine or L-[ring $^{2}H_5$]-phenylalanine 2. Constant infusion of a mixture of U-^{13}C-labelled amino acids obtained from hydrolysis of labelled algal cells.	1. Individual amino acid tracer represents all amino acids and an isotopic 'steady state' exists 2. Material exchanged between the pools or introduced into them mixes completely and instantaneously with that already present 2. Blood flow, tissue uptake of substrate and arterial concentration must all remain constant 3. The venous blood represents the total venous effluent of the tissue	1. Variation is introduced by sampling and errors in measurement of blood flow 2. Indirect measurement of the true precursor, the aminoacyl-tRNA 3. A-V method measures mixed tissue types. For instance, hind limb is a mixture of muscle, skin, fat and bone	Pell *et al.* (1986); Barrett *et al.* (1987); Oddy *et al.* (1987); Lobley *et al.* (1996) Hoskin *et al.* (2003); Savary-Auzeloux *et al.* (2003)

Model used for measuring whole-body protein turnover

Analysis of tracer kinetics for both end-product and tracer loss methods is based on a simple two-pool model (Waterlow *et al.* 1978). In this model (Figure 2.7), amino acids (AA) are considered to be present in the body either as free AA (the substrates of protein synthesis and the products of protein degradation, pool 1) or protein bound AA (pool 2). The rate of amino acids leaving the free amino acid pool per unit time (S and amino acid oxidation) equals the rate entering the free amino acid pool (B and amino acid (dietary + tracer) intake), and equals total flux (Q):

$Q = S + \text{amino acid oxidation} = B + (I + i)$

where amino acid oxidation is measured using either the estimate of CO_2 release in expired air in conjunction with the ^{13}C isotopic enrichment in the precursor pool (in the case of ^{13}C-leucine tracer), or total nitrogen (urea and ammonia) excretion in urine (in the case of ^{15}N-glycine tracer).

This simple model has been widely used for 'steady state' estimations of protein synthesis and degradation. However, the applications have become more diverse and some refinements have been introduced over the years. For further reference in this area the reader is directed to the most recent review in this field by Waterlow (2006).

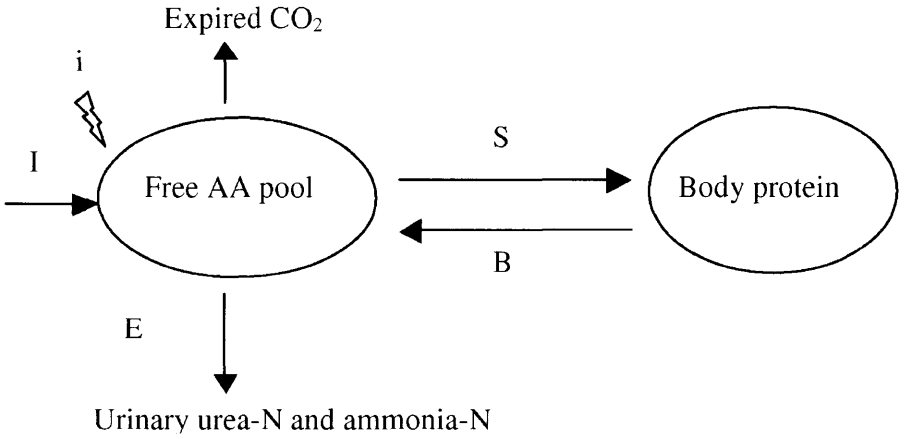

Figure 2.7 A simple model of whole-body amino acid metabolism; the dietary (I) and tracer (i) amino acids enter the free amino acid (AA) pool. From the AA pool, amino acids are assumed to have only two fates: oxidation (carbon to expired CO_2, and nitrogen primarily to urinary urea and ammonia) or use for protein synthesis. S = protein synthesis; B = protein breakdown; I = dietary intake; i = tracer amino acid intake; E = urinary nitrogen excretion

Tissue protein metabolism – with emphasis on the flooding dose method

Arterio-venous balance tracer method

Although surgery is necessary to establish the preparations, with catheters inserted across the arterial input and venous drainage of a defined organ (e.g., gut and liver) or regions (e.g., forearm, leg and skin patch), the net uptake of nutrients, coupled with data on isotope transfer, allows simultaneous calculation of protein synthesis, protein degradation and net amino acid retention. This method requires a constant infusion of a tracer amino acid, along with accurate measurements of arterial and venous isotope enrichments, as well as concentrations of amino acid label uptake across a tissue and blood flow. Both leucine (Pell *et al.* 1986; Oddy *et al.* 1987) and phenylalanine (Barrett *et al.* 1987; Hoskin *et al.* 2003; Savary-Auzeloux *et al.* 2003) have been used as tracers. This method underestimates the total protein synthesis rate because: 1) the amount of intracellular recycling of amino acids; and 2) the ratio between the isotope enrichments of the precursor for protein synthesis (amino acyl transfer RNA) and the enrichment in plasma. Detailed information with regard to calculations of protein synthesis and degradation is given by Davis *et al.* (1999b).

Tracer incorporation method

Tracer incorporation studies have been widely used to measure protein synthesis in tissues. During such studies, a labelled amino acid is administered either by constant infusion or using a 'flooding dose' method, and its incorporation into a protein pool is then quantified (Wagenmakers 1999; Davis and Reeds 2001). With the constant infusion method, a labelled tracer amino acid is infused intravenously at a constant rate until steady-state labelling of the assumed precursor pool for protein synthesis is achieved. This method is particularly well suited to measuring the synthesis of proteins with slow turnover rates, such as those in the muscle (Schaefer *et al.* 1986; Lobley 1990; Davis and Reeds 2001). The constant infusion method has provided most of the data currently available for ruminants.

Accessing the true precursor, amino acyl transfer RNA (tRNA), is in most cases not feasible (Oddy and Neutze 1991). The 'flooding dose' method introduced by Garlick *et al.* (1980) is intended to overcome this problem. This technique involves injecting a large dose of the tracee amino acid together with the tracer amino acid (5 – 50 × the total body free amino acid). Rapid equilibration occurs across all body pools and tracer

enrichment is equalised in the extra-cellular, intra-cellular and amino acyl-tRNA pools (Davis *et al.* 1999a; Waterlow 2006). Furthermore, the flooding dose method enables rapid measurements because it labels the intra-cellular amino acid pool much more rapidly than a continuous infusion of tracer without extra tracee (Davis and Reeds 2001; Waterlow 2006). The flooding dose method is recommended for the measurement of protein synthesis in tissues with rapid turnover rate (Davis and Reeds 2001). The method enables tissue protein synthesis to be determined under acute, non-steady-state conditions of feeding (Davis *et al.* 1996) and during hormone infusion studies (Bark *et al.* 1998).

The fractional rate of tissue protein synthesis (FSR) is calculated as:

$$\text{FSR } (\%/\text{d}) = (E_{t2} - E_{t1}) / \int_{t1}^{t2} Ep(t)dt$$

where E represents the protein bound amino acid enrichment (mol % excess) of the tissue either at time t_1, E_{t1}, or at time t_2, E_{t2}. $\int_{t1}^{t2} Ep(t)dt$ is the area under the curve of the precursor enrichment versus time.

Criteria for selection of an amino acid as a tracer include: a high ratio of protein-bound to intra-cellular-free amino acid content; and similar concentrations of free amino acid in intra-cellular, extra-cellular and blood plasma pools (Oddy and Neutze 1991). An essential amino acid that is neither limiting in the diet nor extensively metabolised to other compounds would be ideal for use as a tracer in tissue protein synthesis studies (Waterlow *et al.* 1978). However, practical matters such as the availability and expense of the tracer and the ease of performing the analysis need also to be considered when choosing a tracer amino acid. The original application of the flooding dose method used leucine as the tracer amino acid (McNurlan *et al.* 1979), but phenylalanine has gradually replaced leucine. Phenylalanine has relatively high solubility and therefore can be easily administered (Garlick *et al.* 1980). Phenylalanine has a smaller pool size than that of leucine, which makes a 'flooding condition more easily achievable' (Waterlow *et al.* 1978). Furthermore, leucine, as opposed to phenylalanine, has been reported to have a stimulatory effect on protein synthesis in some studies (Buse and Reid 1975; Davis and Reeds 2001; Waterlow 2006), although not in others (Garlick *et al.* 1980; Davis *et al.* 1999a). Phenylalanine has distinct advantages as a tracer amino acid.

Clearly, the different approaches described above have provided valuable information about the rates of protein turnover and their functional relationship with nutritional, genotypic and physiological status in farm animals (Table 2.5). However, discrepancies in values in the literature concerning the 'absolute' values of protein synthesis and degradation in response to nutrition and other stimuli exist; these discrepancies may result, to some extent, from the variety of nutritional conditions studied and the different methods, tracers and precursor pools used (Oddy and Neutze 1991; Hoskin *et al.* 2001). These approaches provide approximations of *in vivo* protein synthesis and degradation (Waterlow 2006). In most studies, however, consistency and detection of relative differences are probably more important than absolute accuracy.

Table 2.5 Summary of methods used for measuring protein turnover and some applications

Method	Responses	Reference
Arterio-venous; whole-body protein turnover-L-[1-^{14}C] leucine	Hepatic protein synthesis was maintained at the expense of muscle; muscle protein synthesis contributes to 50 % of whole-body protein synthesis	Pell *et al.* (1986)
Arterio-venous; whole-body protein turnover-[1-^{13}C]leucine	Whole-body and hind-limb protein synthesis increased with food intake	Harris *et al.* (1992)
Constant infusion of [U-^{14}C]phenylalanine and [1-^{13}C]leucine /flooding dose of [^{15}N]phenylalanine	Protein synthesis in muscle, skin and liver increased with intake	Lobley *et al.* (1992)
Arterio-venous; [1-^{13}C] leucine model	Muscle protein synthesis contributed less than 15 % of whole-body protein synthesis; protein degradation had more influence on muscle protein deposition than protein synthesis	Oddy and Lindsay (1986a); Oddy *et al.* (1995)
Flooding dose-L-[ring-d_5]phenylalanine	FSR of the skin and muscle protein was closely related to feed intake; about 18 % of the protein synthesised in skin was deposited as wool protein	Liu *et al.* (1998)
Flooding dose-L-[ring-d_5]phenylalanine	Strains of Merinos selected for and against staple strength differing in protein synthesis in both skin and muscle	Adams *et al.* (2000a)

2.2.4 Factors regulating protein metabolism

The possible interactions between genes, nutrition and hormones and their combined effects on protein metabolism are presented in the following simplified figure (Figure 2.8). Some aspects of genetic variation in protein metabolism have been discussed in detail in Section 2.1, although clearly some genetic factors that regulate protein metabolism also involve interactions between many different pathways. Emphasis will be given here to the extent of protein metabolic changes in response to nutrient intake and some hormones (particularly insulin and insulin-like growth factor I (IGF-I)).

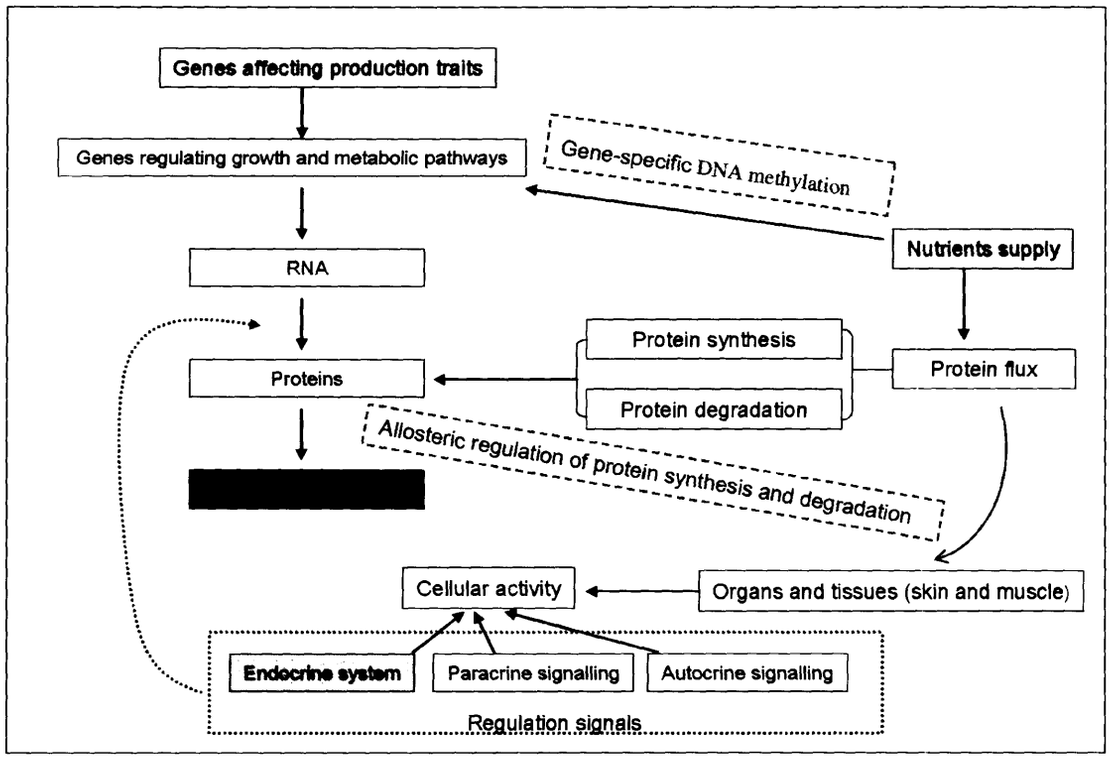

Figure 2.8 Possible components of a mechanism of gene and nutrition interplay on protein turnover

The response of whole-body protein metabolism to changes in nutrient intake is the sum of the different contributions of the various tissues and organs (Waterlow 2006). The contribution of a different tissue or organ depends on the fractional synthesis or degradation rate and its protein mass (Waterlow 2006). Studies have shown that rates of protein synthesis vary widely in individual tissues and organs in ruminants (Table 2.6). These studies also confirm that the liver, rumen and gut have high protein turnover rates

because they are major functional organs playing a vital role in maintenance of body survival (Lobley *et al.* 1992). Although tissues such as muscle and skin have much lower FSRs, their greater protein masses means they contribute significantly to the whole-body protein synthesis and protein gain (Attaix *et al.* 1988; Lobley *et al.* 1992).

Protein metabolism in muscle and skin has been studied because of its importance in meat and wool production (Harris *et al.* 1994; Oddy *et al.* 1995; Liu *et al.* 1998; Adams *et al.* 2000a). The skin is an important intermediate organ for wool growth (Harris *et al.* 1993a; Harris *et al.* 1994; Adams *et al.* 2000b) that accounts for almost 9 % of total body protein, whereas muscle accounts for about 52 % of whole-body protein (MacRae *et al.* 1993). The fractional rate of protein synthesis in muscle is typically 16 % and 33 % of the rate of protein synthesis in the skin of Merino (Liu *et al.* 1998) and Suffolk-cross lambs (Lobley *et al.* 1992), respectively. Therefore, it is reasonable to hypothesise that the response in protein synthesis rate in skin and muscle to changes in feed intake is related to the animal's genetic potential for wool and body growth. This hypothesis was tested in this thesis (See Chapter 5).

Table 2.6 Summary of the relative rates of organ or tissue protein synthesis (FSR) in ruminants

Age and Breed	Diet	Order of FSR	Reference
7-d-old Ile de France × Romanov-Limousin lamb	Commercial milk replacer *ad libitum*	liver > small intestine > abomasum > large intestine > skin > whole-body > skeletal muscle	Attaix and Arnal (1987); Attaix *et al.* (1988)
5-month-old Romney lamb	Fresh ryegrass/clover pasture *ad libitum*	rumen > liver > skin > muscle	Davis *et al.* (1981)
5-month-old Merino lamb	Commercial diet (310 g protein/kg DM)	skin > muscle	Liu *et al.* (1998)
11-month-old Hereford × Friesian heifer	Mixed concentrated diet (60 MJ/d and 650 g protein/d)	small intestine > liver > whole-body > muscle	Lobley *et al.* (1980)
13-month-old lamb	Grass pellets (300 and 900 g/d)	liver > skin > muscle	Lobley *et al.* (1992)
19-month-old Merino sheep	Oaten hay with 10 % lupin seed (9.17 MJ ME/kg DM and 9.6 % protein	small intestine > liver > skin > rumen > muscle	Adams *et al.* (2000a)
Mature Merino ewe	Commercial diet (9.5 MJ/kg DM and 118 g protein/kg DM)	skin > muscle	Liu *et al.* (1999)

Nutrition

Nutritional conditions profoundly affect whole-body protein metabolism and produce differential effects on synthesis and degradation. For example, Harris *et al.* (1992) observed that protein synthesis increased linearly by 23 g /MJ ME between 0.6 M and 1.8 M feed intake in Suffolk-cross lambs. In contrast, protein degradation was apparently unchanged between 0.6 M and 1.2 M but increased at 1.8 M. The increase in protein degradation observed at 1.8 M was lower than the increment in protein synthesis (Harris *et al.* 1992). In further observations on sheep, Liu *et al.* (1995) reported that the daily whole-body protein turnover, synthesis and degradation (g protein/d) during long-term dietary protein excess or deficiency were affected by both dietary protein intake and body protein mass, whereas the fractional rate of turnover (%/d) was mainly influenced by protein intake. There is agreement, however, that during feed deprivation, protein synthesis decreases in sheep (Pell *et al.* 1986; Teleni *et al.* 1986; Oddy *et al.* 1987) and cattle (Lobley *et al.* 1987). This can be considered as an adaptive response which, together with increased protein degradation, assists animals to redistribute their protein reserves and reduce maintenance energy requirement.

Studies with Suffolk-cross lambs (about 7 months old) have shown that improving dietary protein and energy supply (intragastric nutrient infusion or pelleted grass diet) increased the rate of both whole-body protein synthesis and degradation of body proteins, but increased synthesis even more (Harris *et al.* 1992; Liu *et al.* 1995). In 2-year-old Corriedale × Suffolk wethers, protein degradation rate decreased but synthesis rate remained unchanged as protein and energy intake increased (Sano *et al.* 2004). The different responses of protein synthesis and degradation to increased nutrition are probably dependent on breed, body condition, age, type of tracer administrated to animals or feeding regime. On the other hand, the different responses of protein metabolism to increasing feed intake during growth illustrate the complexity of the response in different genotypes of sheep.

Nutrition is also an important regulator of tissue protein metabolism (Lobley 1994). The effect of intake on hind limb protein turnover is shown in Figure 2.9. Protein degradation rate and net protein loss are at their highest in fasting conditions. When the level of intake changes from zero M to 0.5 M, the protein degradation rate seems to decrease sharply (by 60 %), and synthesis rate is also markedly reduced (by 40 %). At the maintenance level of intake, the protein synthesis rate increases and is accompanied

by a further small decrease in degradation rate. With a small increase in intake above maintenance, there is a marked increase in both protein synthesis and degradation rate and a small increase in net protein deposition. The results indicate that, in animals fed at 0.5 – 1.0 M, hind limb protein synthesis is highly labile and protein turnover rate is reduced to low levels.

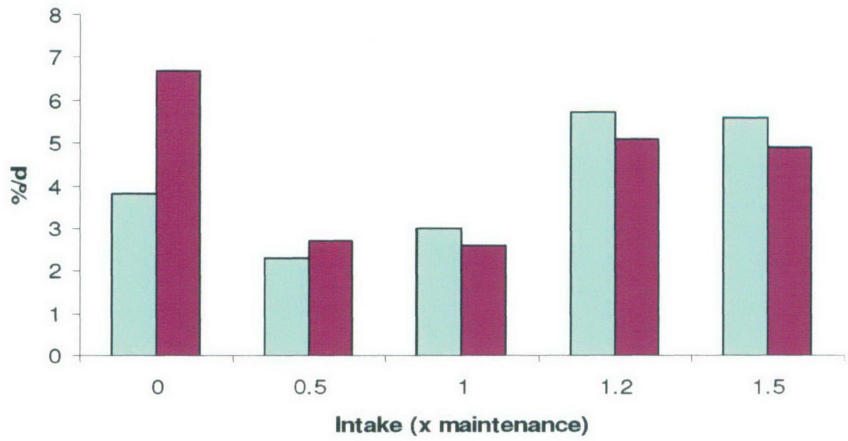

Figure 2.9 Changes in the fractional rates (%/d) of protein synthesis (▯) and degradation (▮) in hind limb peripheral tissues of sheep at varying intakes [a] (Data adapted from different experiments reported by Lobley 1994); [a] Hind limb peripheral tissues include muscle, skin, bone and fat

It has been argued that muscle competes with wool (skin) for amino acids (Cronjé and Smuts 1994), especially in animals that have been selected for increased wool growth (Hatcher 2006). Adams *et al.* (2000b) suggested that different responses of FSR in skin and muscle to changes in feed intake might be a consequence of competition between skin and muscle for available amino acids. Liu *et al.* (1998) illustrate the point with data on the effect of short-term feed intake on protein synthesis in the skin and muscle in Merino lambs. Two groups of Merino lambs differing in liveweight were studied, and fractional rate of protein synthesis in the skin and muscle was determined when the lambs were offered different levels of energy along with different sources of protein. When the animals were under-fed, a greater proportion of ingested nutrients was used to support wool growth at the expense of muscle protein, as indicated by the maintenance of a high rate of protein synthesis in the skin and decreased rate of protein synthesis in muscle. However, when the animals were subsequently well-fed for 4 d (short-term), more nutrients were utilised to support compensatory growth of muscle as shown by the rapid rise of muscle protein synthesis. Even though Liu *et al.* (1998) only examined the

short-term response in protein metabolism in skin and muscle to a change in feed intake, their findings suggest that, by measuring protein synthesis rate, the nature of the response in wool and muscle growth in dual-purpose sheep to short- or long-term nutritional challenges can be identified.

Hormonal control

Whole-body and tissue protein metabolism and its adaptation to various nutritional conditions is subject to endocrine regulation (McDowell and Annison 1989; Lobley 1993a; Oddy 1993; Noguchi 2000). Table 2.7 summarises the principal hormones that regulate metabolism. In ruminants, particular attention has been given to insulin, growth hormone (somatotropin), glucagon, insulin-like growth factor I (IGF-I) and leptin (Bassett 1978; Trenkle 1981; Breier 1999; Daniel *et al.* 2002). These hormones regulate the partitioning of nutrients to specific tissues and nutrient utilisation within those tissues (Trenkle 1981; Oddy and Lindsay 1986b; Lobley 1998).

Table 2.7 Summary of the principal hormones that regulate metabolism (reproduced from Vandehaar 2005)

Hormone	Structure	Source	Regulation	Actions
Insulin: storage hormone	51-AA peptide	β cells of pancreas	↑ rapidly by glucose; AA; ketoacids; propionate; butyrate	↑ glucose use by most tissues; glycogen synthesis; lipogenesis; net protein synthesis ↓ lipolysis
Growth hormone (Somatotropin): anti-obesity production hormone	191-AA peptide	Anterior pituitary	↑ by growth hormone ↓ by somatostatin inversely correlated with nutritional status	↑ IGF-I and thus promotes skeletal muscle growth ↑ net lipolysis ↓ lipogenesis
Insulin-like growth factor-I: anabolic hormone	70-AA peptide	Most tissues, especially liver	↑ by growth hormone ↓ by low insulin long-term; malnutrition positively correlated with nutritional status	↑ DNA synthesis; net protein synthesis; bone elongation, muscle growth
Leptin: 'fuel gauge'	146-AA peptide	Several tissues, especially adipose	↑ by greater body fat mass and greater food intake	↓ feed intake in adults ↑ metabolic rate and thermogenesis in neonates
Cortisol: mobilisation hormone	Steroid	Adrenal cortex	↑ by stress; adrenocorticotrophic hormone	↑ gluconeogenesis, glycogen synthesis; net lipolysis; muscle protein mobilisation ↓ glucose use by tissue
Thyroid hormones: metabolic pacesetter	Small tyrosine-derived compound	Thyroid gland	↑ by cold temperature and thyroid stimulating hormone	↑ metabolic rate within tissues (oxygen consumption)

Insulin is a key hormone involved in regulating the trafficking of amino acids between organs; in particular it facilitates inter-organ transport of amino acids that are used for protein synthesis (Lehninger *et al.* 1993). Plasma insulin concentration is positively correlated with liveweight in sheep and cattle (Speck 1991; Mears 1995). However, insulin-enhanced protein accretion in ruminants is more attributable to inhibition of protein degradation than to stimulation of protein synthesis (Lobley 1998). Oddy *et al.* (1987) found anabolic responses to exogenous insulin occurred in the hind limb of fasted lambs, but did not occur in the hind limb of fed lambs (pre-ruminant). This suggests that, in sheep, unlike in monogastric animals, muscle protein metabolism, and protein degradation in particular, is more responsive to the action of insulin at low feed intake than it is at high feed intake.

Apart from regulating protein metabolism, insulin has a well recognised role in controlling fat and carbohydrate metabolism (Prior and Smith 1982; Weekes 1986; McDowell and Annison 1989). McDowell and Annison (1989) reported that local administration of insulin to hind limb muscle of weaned lambs led to a reduction in concentration of glucose in plasma. Furthermore, intra-arterial insulin infusion to the hind limb of lambs increased fat deposition by 12 % and muscle mass by 5 % (Wolff *et al.* 1989).

It is generally accepted that growth hormone (also termed somatotropin) stimulates cell division, skeletal growth and protein synthesis and promotes release of fatty acids and inhibits fat synthesis in adipose tissue (McDowell and Annison 1989; Zainur *et al.* 1989; Lobley 1998; Schlegel *et al.* 2006). The role of growth hormone in ruminants has been studied using hormone injection. Lambs treated with growth hormone increased their nitrogen retention (MacRae *et al.* 1991) and carcass protein content (Zainur *et al.* 1989; Schlegel *et al.* 2006). The mechanism by which growth hormone leads to increased protein accretion has not been completely resolved. However, there is evidence that exogenous growth hormone administration reduces muscle protein degradation in growing calves (Jois *et al.* 1985) and stimulates muscle protein synthesis in both cattle and sheep (Pell and Bates 1987; Eisemann *et al.* 1989a), as well as whole-body protein synthesis in cattle (Eisemann *et al.* 1989b). The effect of exogenous growth hormone on muscle protein synthesis is mediated through increased production and plasma concentration of IGF-I (McDowell and Annison 1989).

Insulin-like growth factors (IGF-I and IGF-II) play an essential role in regulating animal growth and metabolism (Hossner *et al.* 1997). Plasma IGF-I is positively related to rates of liveweight gain in sheep (Mears 1995; Wylie *et al.* 1997) and cattle (Stick *et al.* 1998) and, specifically, to lean tissue accretion (Douglas *et al.* 1991; Gluckman *et al.* 1991; Lobley 1993a; Oddy and Owens 1996). Systemic administration of IGF-I induced a protein-conserving effect in lambs by increasing protein synthesis and reducing protein degradation in muscle (Douglas *et al.* 1991; Oddy and Owens 1996) and hepatic tissue (Douglas *et al.* 1991).

Plasma IGF-I concentration is sensitive to growth rate (Ellenberger *et al.* 1989; Mears 1995) and to the plane of nutrition (Bass *et al.* 1991; Waterlow 2006). When nutrient supply is above maintenance, circulating IGF-I concentrations are high, permitting high rates of peripheral tissue protein synthesis whereas, during periods of inadequate nutrition, and particularly during fasting, circulating concentrations of IGF-I are reduced, leading to reductions in peripheral tissue anabolism (Breier *et al.* 1986; Bass *et al.* 1991; Wylie 1995). IGF-I might be a useful indicator for selection of body traits. For instance, a divergence in IGF-I concentrations was observed between lines of lambs selected for high and low estimated carcass lean content (Cameron 1992) and for high and low weaning weight (Medrano and Bradford 1991). However, the high sensitivity of IGF-I to dietary energy and protein intake makes IGF-I concentration in young animals, as a predictor of future performance, unreliable (Hossner *et al.* 1997).

Leptin, a hormone produced mainly by adipose tissues, has been implicated in the control of energy balance. Leptin acts on the central nervous system to prioritise the use of available energy among tissues during periods of nutritional insufficiency (Boisclair *et al.* 1994). Leptin is likely involved in mediating the animal's responses to the level of nutrition (Zhang *et al.* 1994; Keisler *et al.* 1999). Previous studies in sheep have shown that plasma leptin concentration was affected by fatness and to a lesser extent by the plane of nutrition (Blache *et al.* 2000; Delavaud *et al.* 2000; Daniel *et al.* 2002). Other studies, mainly in humans and rodents, have shown that leptin was involved in the regulation of energy expenditure (Harvel 1999). Genetic differences in leptin concentrations have been reported between breeds of cattle (Bellmann *et al.* 2004) and between genotypes of meat-producing sheep (Hegarty *et al.* 2006). Whether leptin has potential as a marker of fat-mass in Merino sheep differing in wool-growth potential needs further study.

Responses of wool growth to exogenous growth hormone treatment are inconsistent. Wynn *et al.* (1988) reported growth hormone decreased fleece weight in Merino ewes fed either maintenance or 1.6 M and Johnsson *et al.* (1985) found treatment with growth hormone increased fleece weight in female Merino lambs fed a diet of commercial pellets *ad libitum*. Wynn *et al.* (1988) suggested the variation in response was due to the ability of sheep to repartition nutrients among skin and other tissues. Adams *et al.* (2002) examined the effect of growth hormone on wool growth of Merino and Poll Dorset ewes, when these animals were maintained at pasture in Western Australia. These workers found fleece weight was increased in Merino sheep and decreased in Poll Dorset sheep injected with growth hormone. Adams *et al.* (2002) suggested genotype rather than nutrient supply was the main determinant of the wool growth response to growth hormone.

It has also been demonstrated that short-term (less than 24 h) local infusion of IGF-1 into sheep stimulates protein synthesis in the skin (Harris *et al.* 1993b; Hocking Edwards *et al.* 1995; Lobley *et al.* 1997). However, neither short-term nor long-term (3 wk) local administration of IGF-1 affected wool follicle growth. Similarly, long-term (8 wk) systemic infusion of IGF-I into castrated male yearling sheep did not affect wool growth, despite an increase in plasma concentration of IGF-I (Cottam *et al.* 1992). These observations are consistent with those of Adams *et al.* (1996a; 1996b) who reported systemic IGF-I concentration was not related to wool growth. These studies suggest there may be no effect of IGF-I on wool growth, despite observations that local administration of IGF-I into the skin increased the amount of cysteine and phenylalanine transport to the skin for protein synthesis in Romney sheep (Harris *et al.* 1993a). The lack of response of wool growth to local infusion of IGF-I might be related to specific IGF-binding proteins. For instance, infusion of IGF-I into sheep skin increases local IGF-binding protein production (Hembree *et al.* 1996). The IGF-binding proteins regulate clearance of IFG-I from the circulation and modulate its effect in target tissues (Hossner *et al.* 1997).

Plasma cortisol concentrations were similar in Merino sheep selected for high and low wool growth (Williams *et al.* 1986). The average levels of insulin and growth hormone in plasma were similar for high and low wool-producing sheep (Hough *et al.* 1988). Hough reported significantly lower plasma thyroxine concentrations in Merino sheep selected for high wool growth. Williams (1987) reviewed the effects of insulin, cortisol

and thyroxine on wool production and suggested these three hormones may not contribute significantly to variation in wool growth when the high and low wool-producing sheep are treated alike.

In summary, the rate of whole-body and tissue protein turnover responses to various nutritional conditions through the interaction of numerous hormones. In a fasted animal with a low insulin and IGF-I concentration and a high level of circulating growth hormone, muscle protein synthesis will be depressed and degradation will be elevated. As intake is slowly increased and insulin concentration increases in response, proteolysis will be inhibited, but protein synthesis will be largely unaffected. As intake increases above maintenance, particularly with protein adequate diets, insulin will cease to exert any major additional effect on protein metabolism, but decreased growth hormone and increased IGF-I concentrations will stimulate synthesis and also, but to a lesser extent, degradation (Lobley 1994).

2.2.5 Effect of protein-energy interactions on nutrient partitioning

Energy and protein metabolism interact at almost all levels of biological function. It is not surprising, therefore, that changes in energy intake will give rise to a complex pattern of responses in protein metabolism that depends in part on overall nutritional status and the animal's body condition (Harris *et al.* 1992; Lobley *et al.* 1992; Lobley *et al.* 1994; Chowdhury *et al.* 1997; Savary-Auzeloux *et al.* 2003). Similarly, it is to be expected that the level, and possibly the source of protein ingested would influence energy metabolism (Reeds *et al.* 1985; Lobley 1990; Harris *et al.* 1994).

Factors that contribute to the partitioning of retained energy between protein and fat in growing animals include the animals' stage of maturity and the plane of nutrition relative to maintenance (Webster 1983). The more mature the animal, the greater the proportion of energy retained as fat. A growing animal offered a restricted diet (at slightly below maintenance ME and with enough amino acids) can support protein deposition by mobilising fat (Hovell *et al.* 1983; Webster 1983). When an animal is unable to obtain enough nutrients from the diet, rates of protein and energy metabolism will be reduced; this in turn has adverse consequences for its survival (Chilliard *et al.* 2000).

One important study of the interactions of energy and protein supply on carcass growth and composition was reported by Hegarty *et al.* (1999). The rates of liveweight and carcass protein gain in 4-month-old crossbred lambs (Border Leicester × Merino ewes, Poll Dorset sires) were determined when the lambs were given diets supplying different combinations of energy and protein. When animals' energy intake was low, additional intake of rumen escape protein increased carcass protein gain and skin weight. Liveweight gain, muscle gain and carcass protein gain increased with increased energy intake at all levels of protein intake. The results suggest that protein deposition in lambs is very sensitive to energy supply and efficiency of use of protein for body growth is limited by energy supply (Oddy and Sainz 2002). However, the partial efficiency of energy retention in the carcass does not differ with change in energy intake (Oddy 1997).

The supply of both protein and energy to the small intestine of Merino sheep affects the rate of wool growth. The direction and magnitude of the response to energy intake is dependent upon the particular level of protein given and vice versa. For instance, several authors have reported that when protein was limiting, wool growth was dependent on protein availability, but an increase in ME intake led to a decrease in wool growth. On the other hand, when protein availability was not limiting, an increase in protein supply reduced wool growth, whereas an increase in ME intake stimulated wool growth (Black *et al.* 1973; Kempton 1979; Reis *et al.* 1992). These studies indicated wool growth was maximised with a protein/energy ratio of 12.3 g protein per MJ ME for any given ME or protein intake; the ratio of protein to energy in absorbed nutrients is therefore important, with too little or too much of either component restricting wool growth (Kempton 1979).

Merino genotypes selected for high wool growth in Western Australia had higher protein turnover rate and metabolic rate (Adams and Cronjé 2003; Adams *et al.* 2006a). Merinos selected for higher fleece weight had about 20 % less total body fat than low fleece producers at the same liveweight (Adams *et al.* 2006b). Fogarty *et al.* (2003) also found there was a negative genetic correlation between clean fleece weight and GR site fat depth (GR: Grade Rule for the commercial objective measurement of fat depth, the GR site is 110 mm from the midline over the 12^{th} rib) fat depth (-0.34 ± 0.16). It is uncertain if the key limitation in these high wool growth sheep at different levels of nutrition was the protein needed for wool growth, or dietary energy needed for fat

deposition. To determine the limiting factors, it seems logical to investigate how and to what extent the efficiency of utilisation of ingested energy and protein in Merinos is changed by genetic selection for superior wool growth.

2.2.6 Conclusion

The significance of protein and energy metabolism and the regulation of nutrient utilisation in ruminants were reviewed in this section. The energy expenditure associated with extensive turnover of protein in tissues implies protein and energy metabolism are inextricably linked. These processes are significantly affected by the nutritional and hormonal status of the animal. There is some evidence that there is 'competition' between skin and muscle for available nutrients, especially energy and protein. Direct measurements of protein synthesis and degradation in the whole-body and in tissues such as muscle and skin, and whole-body energy expenditure rate can provide a better understanding of the processes that influence tissue protein gain and loss, and wool growth, in Merino sheep.

Most of the methods currently used to determine protein turnover and some of the methods used to estimate energy expenditure rate in different dietary contexts are tracer-based. These tracer techniques might not necessarily yield absolute or true values for protein and energy metabolism. However, most estimates of protein turnover derived from the flux of a tracer amino acid and energy expenditure rate derived from the flux of CO_2 are used on a comparative basis for one species within the same experiment. Biological responses/trends observed within an experiment can usually be interpreted with confidence, although this confidence may not apply to estimates absolute turnover rate across experiments.

2.3 Summary of literature review

An understanding of current knowledge of ruminant protein and energy metabolism and the methods available to quantify them (Section 2.2) is an essential prerequisite for conducting studies to obtain a better understanding of the metabolic processes and physiological consequences involved in genetic selection for wool growth or meat production (Section 2.1).

This review has illustrated that Merino sheep have been successfully selected for many traits including wool and meat production. Although the economic importance of genetic selection for superior wool and meat production traits is clear, how the underlying physiological processes are changed by breeding remains unclear, with only limited research available. Without knowing the underlying physiological processes on which genetic selection acts, genetic improvement through selection is essentially a 'black box technique'. Even though genetic selection based on different multi-trait indices will almost certainly generate improvements in the desired traits via somewhat different physiological mechanisms, there are likely to be major pathways in common. Protein and energy metabolism are the obvious starting place for physiological studies of the consequences of selective breeding. Studies on protein and energy metabolism at the whole-body and tissue (skin and muscle) level, along with the related traits (wool characteristics and body traits), might offer farm managers the opportunity to identify and eventually avoid unfavourable side-effects of selection, such as those found by Adams *et al.* (2006b).

Trials based on the studies reviewed above are presented in the following experimental chapters (Chapters 3 – 7).