A partial cDNA sequence of the ovine insulin receptor gene: evidence for alternative splicing of an exon 11 region and for tissue-specific regulation of receptor isoform expression in sheep muscle, adipose tissue and liver

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Abstract

Insulin is as integral and important to the management of metabolism in ruminants as it is in non-ruminants. The suggestion of a lowered ruminant sensitivity and/or responsiveness to insulin may relate more to the insulin receptor than to the hormone itself. We screened an ovine cDNA library using degenerate primers and polymerase chain reaction (PCR) to detect and sequence a cDNA portion corresponding to exons 10, 11 and 12 of the human insulin receptor gene in which a 36 base pair (bp) segment (exon 11) is alternatively spliced to produce two distinct receptor isoforms differing in functional characteristics including binding affinity for insulin. The ovine cDNA segment (nucleotides 671 to 770) displayed 84, 84, and 78% nucleotide homology to equivalent segments from the human, rhesus monkey and rat respectively. Reverse transcription PCR (RT-PCR) of selected tissues (liver, m. longissimus dorsi, m. rectus capitis and omental, perirenal and subcutaneous fats) taken at slaughter from three male, pure Dutch Texel lambs (experiment 1) and five male Texel-Greyface crossbred lambs (experiment 2) revealed two mRNA products in each tissue (including spleen; experiment 2 only) corresponding to cDNAs of molecular sizes 161 and 197 bp – a difference of 36 bp. Sequence alignment showed the 36 bp segment to be homologous to the alternatively spliced exon 11 region of

the human insulin receptor gene and to be highly conserved with that from other species. The abundance of the exon 11⁺ isoform in the purebred Texel genotype was significantly higher in liver than in perirenal fat and rectus capitis and longissimus dorsi skeletal muscles ($P < 0.05$) and higher also than in subcutaneous and omental fats ($P < 0.01$). There was, however, no difference in the abundance of the exon 11⁺ isoform between the individual muscle and fat depots in this sheep genotype. The abundance of the exon 11⁺ isoform in the crossbred Texel genotype was significantly higher in liver ($P < 0.05$) than in the muscles (rectus capitis, $P < 0.05$; longissimus dorsi, $P < 0.001$), all three fats ($P < 0.001$) and spleen ($P < 0.001$). In the crossbred genotype, the abundance of the exon 11⁺ isoform was higher in skeletal muscle than in all three fat depots ($P < 0.001$), in which the isoform abundance was similar. Altered ratios of expression of the two products of this alternative splicing event could determine tissue sensitivity and/or responsiveness to insulin and provide a mechanism for the management of nutrient partitioning and nutrient utilisation between tissues which is fundamental to the growth of tissues and manipulation of carcass characteristics in meat-producing animals.

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Introduction

Little glucose is absorbed from the gut of domesticated ruminants (cattle, sheep, goats) which feed largely on forage diets occasionally supplemented with variable levels of energy concentrates such as barley or maize and protein concentrates such as extracted soya bean, sunflower seed or fish meals. Most glucose entering the rumen directly in the diet or released by intra-ruminal digestion of complex forage carbohydrate is metabolised to a number of short-

chain fatty acids (acetic, propionic, butyric and valeric) by a mixed microbial and protozoal population in the rumen. Only when energy concentrates are fed in large amounts, or in protected form, do significant quantities of glucose survive ruminal breakdown and become available for post-ruminal (i.e. ileal) absorption (Nocek & Tamminga 1991).

Despite the paucity of glucose absorbed from the gut of the traditionally-fed ruminant, the ruminant animal has needs for glucose similar to those of non-ruminants (i.e. an

obligatory requirement for brain function and a variable requirement for the conventional pathways of cellular metabolism) and glucose demand in ruminants is met largely by hepatic gluconeogenesis. Blood glucose concentration (typically 3–6 mM) in ruminants is regulated by the actions of the same counter-regulatory hormones (insulin and glucagon) as in non-ruminants and glucose is moved into both ruminant muscle (Hocquette *et al.* 1995) and adipose cells (Hocquette *et al.* 1996) by the same insulin-responsive glucose transporter (GLUT 4) as a consequence of insulin binding to receptors in the cell membrane. While the principal substrate for fatty acid synthesis in ruminants is acetate of ruminal, rather than glycolytic origin, glucose provides both the glycerol component for triacylglycerol synthesis and the NADPH required for the reductive steps of fatty acid synthesis. The interrelated metabolic processes of gluconeogenesis, glycolysis, fatty acid synthesis, lipolysis and protein synthesis and catabolism are all under the partial control of insulin in ruminants as in non-ruminants.

Overall, therefore, insulin is as integral and important to the management of metabolism in ruminants as in non-ruminants. However, ruminants are often regarded as being less sensitive and/or responsive to insulin than non-ruminants (Vernon *et al.* 1985, Sasaki 1989). Sensitivity and responsiveness to a hormone are related to the hormone's interaction with its receptor and/or to post-receptor events (Kahn 1978) and the affinity of a receptor for its hormone is likely to be fundamental to both sensitivity and responsiveness.

The affinity of the insulin receptor for insulin has been shown by classical hormone-binding studies to differ between pig breeds (Camara & Mourot 1996) and between tissues in humans (Bolinder *et al.* 1983, Kotzke *et al.* 1995) and in the ruminant (McGrattan *et al.* 1997, Wylie *et al.* 1998). Differences in insulin binding affinity (K_d) between liver, muscle and fat of sheep (Wylie *et al.* 1998) may underlie altered tissue responsiveness to insulin, but it is unclear how or why differences in insulin binding affinity between tissues arise. One possibility is manipulation of the ratio of expression of receptor isoforms, of innately different affinity for insulin, generated by alternative splicing (Breitbart *et al.* 1987) of a single insulin receptor gene as shown in humans (Moller *et al.* 1989, Seino & Bell 1989), rats (Goldstein & Dudley 1990) and the rhesus monkey (Huang *et al.* 1994). In each of these species, the insulin receptor is expressed as two isomeric products (A and B) of two mRNAs (exon 11⁺ and exon 11⁻) which differ only in the presence or absence of a 36 base pair (bp) exon 11 region. Importantly, the A and B isoforms of the human insulin receptor differ in affinity for insulin (A>B) when expressed in cultured cell lines (Mosthaf *et al.* 1990).

Differences in affinity for insulin between tissues may influence the partitioning of nutrients between tissues (e.g. muscle and fat) and affect their relative rates of growth.

This has potential, but clear, implications for the regional distribution of fat in humans and animals and for the manipulation of carcass composition (e.g. fat vs lean) in meat-producing animals.

Materials and Methods

Isolation of ovine insulin receptor cDNA and demonstration of two alternatively spliced mRNAs

Sense and antisense degenerate PCR primers encoding amino acids highly conserved in human, rat and mouse insulin receptor exon 10 and exon 12 respectively were used to screen an ovine omental adipose cDNA library (a gift from the Hannah Research Institute, Ayr, Scotland, UK). The sense primer (S1) consisted of a 20-mer with the sequence 5'-CARAARCAMAAYCARTCIGARTA-3' encoding the exon 10 amino acid sequence QKHNQSEY, while the antisense primer (AS1) was a 20-mer with the sequence 5'-AARCCITGGACICARTAYGCIAT-3' encoding the exon 12 amino acid sequence KP-WTQYAI.

PCR was performed in a 25 µl reaction volume using Ready-to-Go PCR beads (Pharmacia Biotech, St Albans, Herts, UK) containing 200 µM of each dNTP, 1.5 mM MgCl₂, 10 mM Tris, pH 9.0, 50 mM KCl and 0.5 units of AmpliTaq DNA polymerase, 5 µl ovine cDNA and 2 µl (40 pmol) of each sense (S1) and antisense (AS1) degenerate primer. Each cycle consisted of denaturation at 95 °C (1 min), annealing at 52 °C (1 min) and DNA polymerisation at 72 °C (2 min) in an automated thermal cycler (Model 2400, PE Biosystems, Warrington, Cheshire, UK). The final cycle included an incubation at 72 °C for 7 min.

Amplified PCR products were analysed by gel electrophoresis using 2% (w/v) high resolution agarose (Sigma Chemical Co., Poole, Dorset, UK) in 1 × Tris acetate buffer (TAE; 0.089 M Tris, 0.089 M acetic acid, 0.002 M EDTA, pH 8.0). Appropriate PCR products were purified by 'band-stab' PCR (Bjorson & Cooper 1992) and both strands were sequenced in triplicate using an automated ABI 373 sequencer (PE Biosystems). The nucleotide sequence and deduced amino acid sequence data were compared with sequences deposited in the EMBL and SWISSPROT databases using the BLASTn and BLASTx programs (Altschul *et al.* 1990).

Investigation of tissue-specific expression of alternatively spliced insulin receptor isoforms in sheep

Experiment 1 Three male Dutch Texel lambs were fed a diet of dried-grass nuts available *ad libitum* from weaning until slaughter at 8 months of age (slaughter weight = 47 ± 0.5 kg). Samples of liver (L), m. longissimus dorsi (LD), m. rectus capitis (RC) and subcutaneous (SCF),

(A)

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TCAGTTCGGAGTATGGAGGAGTCTGCTGGCGAATGCTGCTCCTGCCCGAAGACCGACTCT 60
S V R S M E E S A G E C C S C P K T D S 20

CAGATCCTCAAGGAGCTGGAGGAGTCTCGTTTCAGGAAAACGTTTGAAGATTACCTGCAC 120
Q I L K E L E E S S F R K T F E D Y L H 40

AACGTGGTTTTTCATCCCCAGGCCTTCGAGGAAACGCAGAGCCCTTGGTGACGTGGGGAAT 180
N V V F I P R P S R K R R A L G D V G N 60

GTGACAGCAGCCGTGCCCACTGTTCTGGGGTTCCCCAATAGCTCCTCCACCAGCACACCC 240
V T A A V P T V L G F P N S S S T S T P 80

ACAAGCTCAGAGGAGCACAGAACC 264
T S S E E H R T 88

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(B)

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TCAGTTCGGAGTATGGAGGAGTCTGCTGGCGAATGCTGCTCCTGCCCGAAGACCGACTCT 60
S V R S M E E S A G E C C S C P K T D S 20

CAGATCCTCAAGGAGCTGGAGGAGTCTCGTTTCAGGAAAACGTTTGAAGATTACCTGCAC 120
Q I L K E L E E S S F R K T F E D Y L H 40

AACGTGGTTTTTCATCCCCAGAAATCATCTTCAGGCCCTGTTGCCGAGGACGCTAGGCCT 180
N V V F I P R K S S S G P V A E D A R P 60

TCGAGGAAACGCAGAGCCCTTGGTGACGTGGGGAATGTGACAGCAGCCGTGCCCACTGTT 240
S R K R R A L G D V G N V T A A V P T V 80

CTGGGGTTCCCCAATAGCTCCTCCACCAGCACACCCACAAGCTCAGAGGAGCACAGAACC 300
L G F P N S S S T S T P T S S E E H R T 100

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Figure 1 Partial nucleotide and deduced amino acid sequences of isoforms of the ovine insulin receptor gene. (A) Exon 11⁻ (nucleotides 1–141: exon 10; 142–264: exon 12) and (B) exon 11⁺ (nucleotides 1–141: exon 10; 142–177: exon 11; 178–300: exon 12). Nucleotide number and amino acid residue number are shown on the right. The alternatively spliced exon 11 region (base pair numbers 142 to 177 and amino acid numbers 48 to 59) is shown in bold and underlined in (B).

omental (OF) and perirenal (PF) fats were collected as soon as possible after captive-bolt slaughter and were immediately frozen in liquid nitrogen prior to storage at -70°C . Total RNA was isolated from 20 mg of the appropriate tissue using TRIzol reagent (Gibco BRL, Paisley, Strathclyde, UK) according to the manufacturer's protocol.

Experiment 2 Five male crossbred (0.75 Texel:0.25 Greyface) lambs were grazed on a ryegrass pasture from weaning until 5 months of age (mean liveweight of 32.5 ± 2 kg) and then fed a 24-h wilted grass silage available *ad libitum* until slaughter at 8 months of age (slaughter weight = 36.0 ± 4.6 kg). Samples of L, LD, RC, SCF, OF, PF and spleen (S) were collected as soon as possible after captive-bolt slaughter and were immediately frozen in liquid nitrogen prior to storage at -70°C . Total RNA was isolated from 20 mg of the appropriate tissue

using TRIzol reagent (Gibco BRL) according to the manufacturer's protocol.

Isolation and reverse transcription of total RNA

Reverse transcription-PCR (RT-PCR) was performed essentially according to Sesti *et al.* (1994) using sense and antisense oligonucleotides designed against the determined ovine specific insulin receptor nucleotide sequence (EMBL accession no. Y16092). The primers were designed to yield products of 161 and 197 bp for the A and B insulin receptor isoforms respectively. First strand cDNA synthesis was performed using 200 units Superscript II reverse transcriptase (Gibco BRL) and total RNA (12 μl) from the appropriate tissue. The RNA was denatured at 65°C for 5 min along with 2 pmol ovine species-specific antisense primer CA1 (5'-GTGGGCACGGCTGCTGTCAC-3' corresponding to exon 12 amino acids

Ovine	671	-----SVRSMEESAGECCSCPKTDSQ	691
Human	671	-----NQSEY-----	691
Monkey	671	-----NQSEY-D-----N--	691
Rat	673	-----NQSEYDD--S-----	693
Mouse	673	-----NQSEYDD--S-----	693
Ovine	692	ILKELEESSFRKTFEDYLHNVVFI	721
Human	692	-----V--T--	721
Monkey	692	-----V--T--	721
Rat	694	-----V--T--	723
Mouse	694	-----V--T--	723
Ovine	722	GPVAEDARPSRKRRALGDVGNVTA	751
Human	722	-TG---P-----S-----V----A	751
Monkey	722	-TG---P-----S-----V----A	751
Rat	724	-NG---T-----S-EE-----TT--LP	753
Mouse	724	-NG---S-----S-EE-----TTL-LP	753
Ovine	752	GFPNSSSTSTPTTSSEEHRT	770
Human	752	A---T---V---PEEHRPF	781
Monkey	752	A---T---V---PEEHRPF	781
Rat	754	D---I---IA---HEEHRPF	783
Mouse	754	D---V---IV---QEEHRPF	783

Figure 2 A comparison of the predicted amino acid sequence of the ovine insulin receptor (exons 10 through 12) with that of other species (numbered according to Ebina *et al.* 1985). The sequence of the exon 11 region in mouse was obtained through Genbank accession number L42997. The difference in numbering of the amino acid residues in rat and mouse compared with sheep, human and monkeys is due to two additional residues at positions 547 and 548 which are unique to the rat and mouse.

VTAAVPT in Fig. 1). To the denatured RNA was added 4 µl first-strand 250 mM Tris–HCl buffer (pH 8.3 at room temperature) containing 375 mM KCl, 15 mM MgCl₂ (Gibco BRL), 5 mM dithiothreitol and 0.5 mM dNTP and the mixture was incubated at 42 °C for 50 min. Two control reverse transcriptions either (a) without reverse transcriptase or (b) without RNA template were performed in parallel with the samples.

An aliquot (2–5 µl) of each RT reaction mixture was subjected to 30 cycles of PCR amplification under con-

ditions known to prevent the formation of heteroduplexes (Norgren *et al.* 1993). Each reaction used 20 pmol ovine species-specific sense primer CS1 (5′-TCCTGCCCCG AAGACCGACTC-3′) and the CA1 antisense primer which was used for reverse transcription – 200 µM dNTP, 1.5 mM MgCl₂, 10 mM Tris, pH 8.3, 50 mM KCl, and 0.5 units of AmpliTaq DNA polymerase. Each reaction was amplified for 30 cycles and each cycle consisted of denaturation at 95 °C (30 s), annealing at 60 °C (30 s) and DNA polymerisation at 72 °C (30 s) in an automated thermal cycler. The final cycle included an incubation at 72 °C for 7 min. Following amplification, 10 µl of each reaction mixture was analysed by gel electrophoresis as described previously.

The percentage abundance of the alternatively spliced insulin receptor PCR products in individual tissues was calculated by scanning densitometry using Phoretix 1D Advanced (version 3.01) computer software (Phoretix International Ltd, Newcastle, UK). Statistical analysis for tissue differences was by ANOVA and the least significant differences between tissues were calculated using the Student’s *t*-test. The statistical package used was Genstat 5 (NAG 1993).

Results

PCR amplification using the degenerate primers S1 and AS1 yielded two discrete PCR products of 293 bp and 329 bp, which were consistent with the sizes of products expected from alternative splicing of the ovine insulin receptor in line with that of the human insulin receptor. The nucleotide sequences and deduced amino acid open reading frames of these products are shown in Fig. 1. The two cDNAs differ only in the inclusion of a 36 bp sequence encoding 12 amino acids in the larger product (Fig. 1). Database homology analyses of these

Figure 3 RT-PCR analysis for detection and determination of the relative abundance of exon 11 splice variants from a variety of tissue depots from purebred Texel male lambs (experiment 1). Ovine cDNA (from L, LD, RC, SCF, OF and PF) were used as templates, with forward (CS1) and reverse (CA1) primers designed to bind within exons 10 and 12 respectively. The exon 11 splice variant was detected due to products differing by 36 bp in each of the lanes. Molecular weight marker VI (Boehringer Mannheim, Lewes, E. Sussex, UK) is shown on the right. Thirty cycles of PCR were performed as described in Materials and Methods and amplified products were resolved on a 2% (w/v) agarose TAE gel and visualised under UV light. The relative abundance of exon 11⁺/11[–] products was determined by scanning densitometry.

Figure 4 RT-PCR analysis for detection and determination of the relative abundance of exon 11 splice variants from a variety of tissue depots (A, liver; B, rectus capitis; C, omental fat; D, spleen) from crossbred Texel-Greyface lambs (experiment 2). Ovine cDNA from each tissue was used as a template, with forward (CS1) and reverse (CA1) primers designed to bind within exons 10 and 12 respectively. The exon 11 splice variant was detected due to products differing by 36 bp in each of the lanes. Molecular weight marker VI (Boehringer Mannheim) is shown on the left. Thirty cycles of PCR were performed as described in Materials and Methods and amplified products were resolved on a 2% (w/v) agarose TAE gel and visualised under UV light. The relative abundance of exon 11⁺/11⁻ products was determined by scanning densitometry.

sequences confirmed that the products encoded part of the insulin receptor exon 10 and exon 12 and that the additional 36 bp in the larger sequence was derived from exon 11.

Nucleotide and deduced amino acid sequences of the alternatively spliced ovine insulin receptor region encoding exon 10 (partial), exon 11 and exon 12 (partial) exhibited high degrees of homology with the corresponding regions from rhesus monkey (84% and 87% respectively), human (84% and 86% respectively) and rat (78% and 78% respectively) (Fig. 2). The results of RT-PCR expression analyses and gel electrophoresis of alternatively spliced insulin receptor cDNA amplification products from some of the tissues from the male Dutch Texel lambs and from the male Texel-Greyface crossbred lambs are presented in Figs 3 and 4 respectively. All tissues gave two discrete bands representing the A and B isoforms and differing in size by exactly 36 base pairs.

Tables 1 and 2 show the relative abundance of A isoform and B isoform mRNA transcripts in liver, muscle, fat and spleen (Table 2 only) from the purebred Texel and crossbred Texel lambs respectively. The relative abundance of the alternatively spliced receptor isoforms differed between liver and all other tissues sampled (L vs SCF, OF: $P < 0.01$; L vs PF, RC, LD: $P < 0.05$; $n = 3$, S.E.M. = 2.055) in the purebred Texel lambs, but there were no significant differences in the percentage abundance of individual receptor isoforms between the skeletal muscles or between any of the fat depots or overall, between muscles and adipose tissues for this genotype. In all tissues, the B isoform was the dominant isoform, ranging from 70.21% of total receptor mRNA in subcutaneous fat to 80.15% of total receptor mRNA in liver.

The relative abundance of the alternatively spliced receptor isoforms differed between liver and all other tissues analysed (L vs RC: $P < 0.05$; L vs all other tissues: $P < 0.001$; $n = 5$, S.E.M. = 1.535) in the crossbred Texel-Greyface genotype lambs. The abundance of the B isoform mRNA transcript was significantly higher in liver compared with rectus capitis skeletal muscle ($P < 0.05$) and all other tissues ($P < 0.001$). Spleen had the highest abundance of the A isoform mRNA transcript ($P < 0.001$). Both skeletal muscles (rectus capitis and longissimus dorsi) had a significantly higher abundance of the B isoform transcript compared with any of the three adipose tissue depots ($P < 0.001$). The relative abundance of the B isoform mRNA transcript was higher in rectus capitis than in longissimus dorsi ($P < 0.05$) but there was no difference in the abundance of this transcript between any of the three adipose tissue depots.

Table 1 Percentage abundance of the two alternatively spliced insulin receptor mRNA transcripts (exon 11⁻ (A) and exon 11⁺ (B)) in a variety of tissue depots (L, RC, LD, SCF, OF and PF) in purebred Texel male lambs

	L	RC	LD	SCF	OF	PF
A (exon 11 ⁻)	19.85 ^a	27.27 ^b	26.58 ^b	29.79 ^b	29.05 ^b	28.69 ^b
B (exon 11 ⁺)	80.15 ^a	72.73 ^b	73.42 ^b	70.21 ^b	70.95 ^b	71.31 ^b

Different superscripts within a row indicate tissue-specific differences ($P < 0.05$ to $P < 0.001$; S.E.M. = 2.055, $n = 3$).

Table 2 Percentage abundance of the two alternatively spliced insulin receptor mRNA transcripts (exon 11[−] (A) and exon 11⁺ (B)) in a variety of tissue depots (L, RC, LD, SCF, OF, PF and S) in male Texel-Greyface lambs

	L	RC	LD	SCF	OF	PF	S
A (exon 11 [−])	19.58 ^a	23.02 ^b	28.28 ^c	46.13 ^d	46.18 ^d	47.96 ^d	30.25 ^e
B (exon 11 ⁺)	80.42 ^a	76.98 ^b	71.72 ^c	53.97 ^d	53.82 ^d	52.04 ^d	69.75 ^e

Different superscripts within a row indicate tissue-specific differences ($P<0.05$ to $P<0.01$; S.E.M.=1.535, $n=5$).

Discussion

In this study, we used degenerate PCR primers to screen an ovine cDNA library to determine whether alternative splicing of the insulin receptor occurs in sheep (and, by implication, in ruminant animals in general) as it does in non-ruminants. The detection of two discrete PCR products differing by exactly 36 base pairs in all the tissues sampled, confirmed that alternative splicing of the insulin receptor gene does indeed occur in sheep. Construction of two species-specific primers allowed the identification and quantification of the two mRNA transcripts of the alternatively spliced exon 11 variants, and differences in the percentage abundance of the two mRNA transcripts between a selection of insulin-sensitive tissues provides additional evidence for tissue-specific regulation of insulin receptor alternative splicing in sheep.

In both the lamb genotypes used in the study, liver contained a higher proportion of the lower affinity exon 11⁺ isoform compared with skeletal muscle and adipose tissue. The actual physiological significance of this difference in expression of the two alternatively spliced receptor isoforms and in insulin binding affinity between liver and other tissues in ruminants (McGrattan *et al.* 1997, Wylie *et al.* 1998) is unknown, but it is considered significant that liver is exposed to portal insulin concentrations which are typically 2- to 3-fold higher than peripheral insulin concentrations. The predominance of the lower affinity exon 11⁺ insulin receptor transcript in liver is consistent with the lower overall insulin binding affinity of this tissue in both cattle (McGrattan *et al.* 1997) and sheep (Wylie *et al.* 1998) and may allow liver to respond appropriately to fluctuating, and occasionally high, portal insulin concentrations while accommodating and permitting insulin's metabolic effects and providing significant hepatic clearance of insulin.

Although no differences in expression of alternatively spliced insulin receptor isoforms between peripheral tissues were demonstrated for the purebred Dutch Texel lambs in experiment 1, significant differences were detected both between tissues (skeletal muscle vs adipose tissue) and within tissue types in the cross-bred Texel lambs in experiment 2. It is assumed that differential distribution of the alternatively spliced insulin receptor isoforms between tissues in the cross-bred lamb genotype affects overall

insulin affinity of the respective tissues and may have implications for the partitioning of nutrients between individual skeletal muscles and adipose tissue depots in these lambs. In related studies, Carson (1997) has demonstrated strong genotypic influences on carcass quality characteristics in lambs. Specifically, increasing the proportion of Texel genes (0; 0.50; 0.75; 1.0) in a Texel-Greyface lamb population significantly increased the proportion of recoverable carcass (dressing proportion), the carcass conformation classification (grading) and the lean content of the carcass, while reducing the proportions of both intermuscular and subcutaneous fat. Reducing the fat content and improving the lean content of lamb carcasses is regarded as being of crucial importance to the future success of the extensive and intensive UK sheep industries and the genotypic influences on carcass traits shown by Carson (1997) may be related, through effects on nutrient partitioning, to primary causes such as tissue responsiveness to individual metabolic hormones.

Previous demonstrations of differences in insulin binding affinity between muscle and adipose depots of cattle- and of sheep in our laboratory (McGrattan *et al.* 1997, Wylie *et al.* 1998) and of differences in the insulin binding affinity of the human A and B alternatively spliced insulin receptor isoforms when expressed in cultured cell lines (Mosthaf *et al.* 1990), combined with indications of tissue-specific expression of the human and rat A and B isoforms (Seino & Bell 1989, Mosthaf *et al.* 1990), suggest that alternative splicing of the insulin receptor may be one mechanism by which the responsiveness of insulin-sensitive tissues to insulin is modulated in ruminant and non-ruminant animals. Alteration of the ratio of expressed receptor isoforms and, hence, the overall affinity of individual tissues for insulin may influence the way in which nutrients are partitioned to, and ultimately utilised by, competing tissues (e.g. muscle, fat) and even by separate tissues of the same type (e.g. omental vs perirenal vs subcutaneous fat). It is interesting to speculate that, while the affinity of individual tissues for insulin may be specified genetically through dictation of receptor isoform ratio, it might also be manipulated by dietary-induced alteration of receptor isoform ratios through differential down-regulation of the two isoforms by diet-induced hyperinsulinaemia.

This is the first demonstration of alternative splicing of the insulin receptor gene in ruminant animals and of tissue-specific expression of the two insulin receptor gene mRNA transcripts in ruminant tissues. Identification of the nucleotide sequence encoding the exon 11 region confirms that this alternative splicing event is essentially identical to that demonstrated in humans (Ebina *et al.* 1985, Ullrich *et al.* 1985), rats (Goldstein & Dudley 1990), and the rhesus monkey (Huang *et al.* 1994), and makes it possible to investigate breed and diet effects on tissue-specific expression of the insulin receptor mRNA variants in sheep and to use receptor isoform ratio as a potential marker of genotypically-related carcass traits.

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